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Strugnell et al.

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(54) **ENGINEERED INFLUENZA ANTIGENIC
POLYPEPTIDES AND IMMUNOGENIC
COMPOSITIONS THEREOF**

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CPC **C07K 14/11** (2013.01); **A61P 31/16**
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None
See application file for complete search history.

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ABSTRACT

The present invention provides, among other things, a novel
and improved method for generating "mosaic" influenza
antigenic polypeptides including hemagglutinin (HA) and
neuraminidase (NA) polypeptides based on unique combi-
nation of epitope patterns that maximize exposure to
epitopes present across multiple HA or NA sequences and
therefore improved influenza strain coverage. In particular,
the present invention provides engineered influenza B
hemagglutinin (HA) polypeptides that are comprised of
novel combinations of protective epitopes and antigenic
regions from multiple influenza B viral strains. Such engi-
neered HA polypeptides have improved properties over HA
polypeptides developed through conventional approaches
that rely on consensus alignments of viral sequences.

19 Claims, 23 Drawing Sheets

Specification includes a Sequence Listing.

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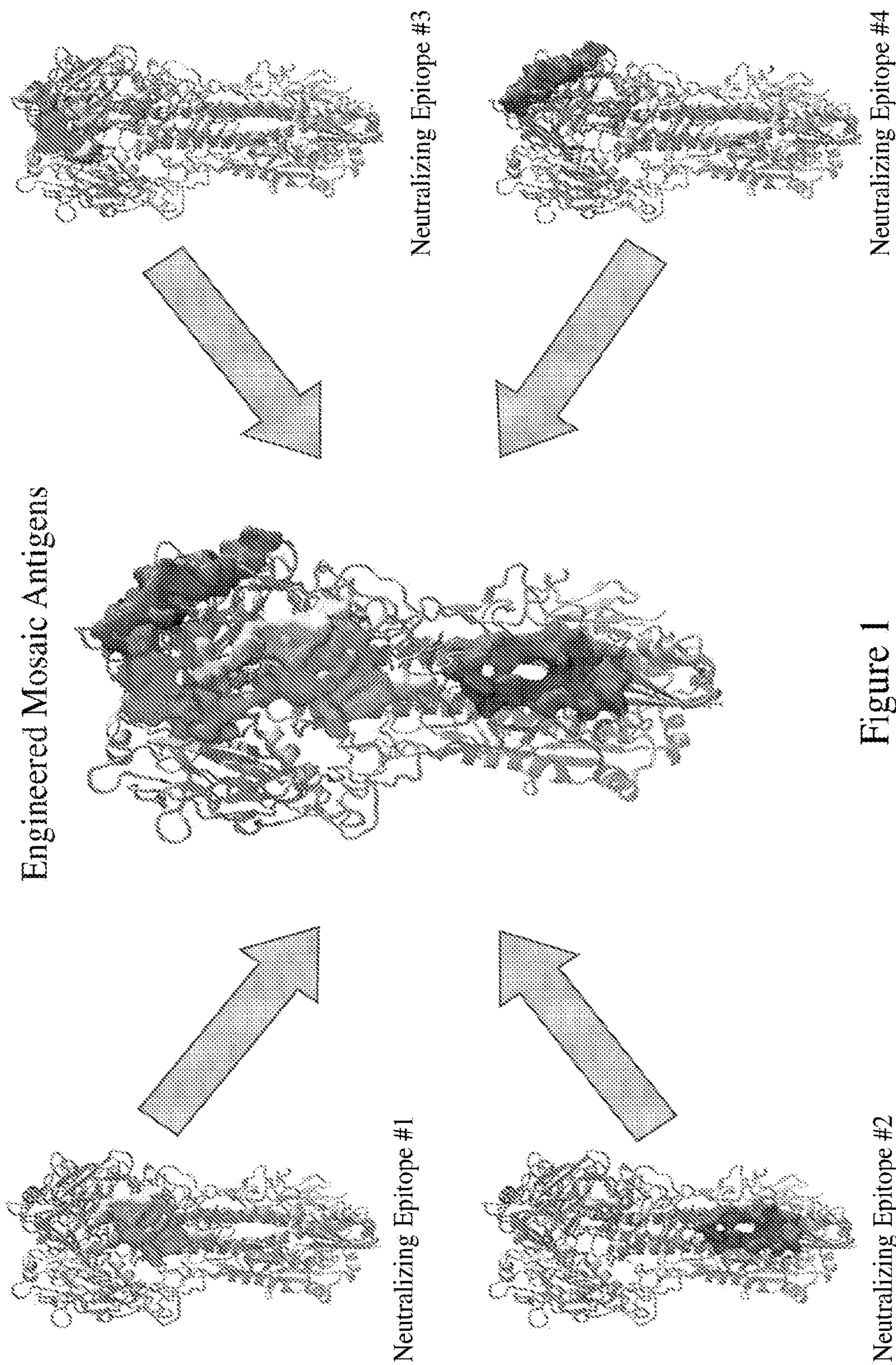


Figure 1

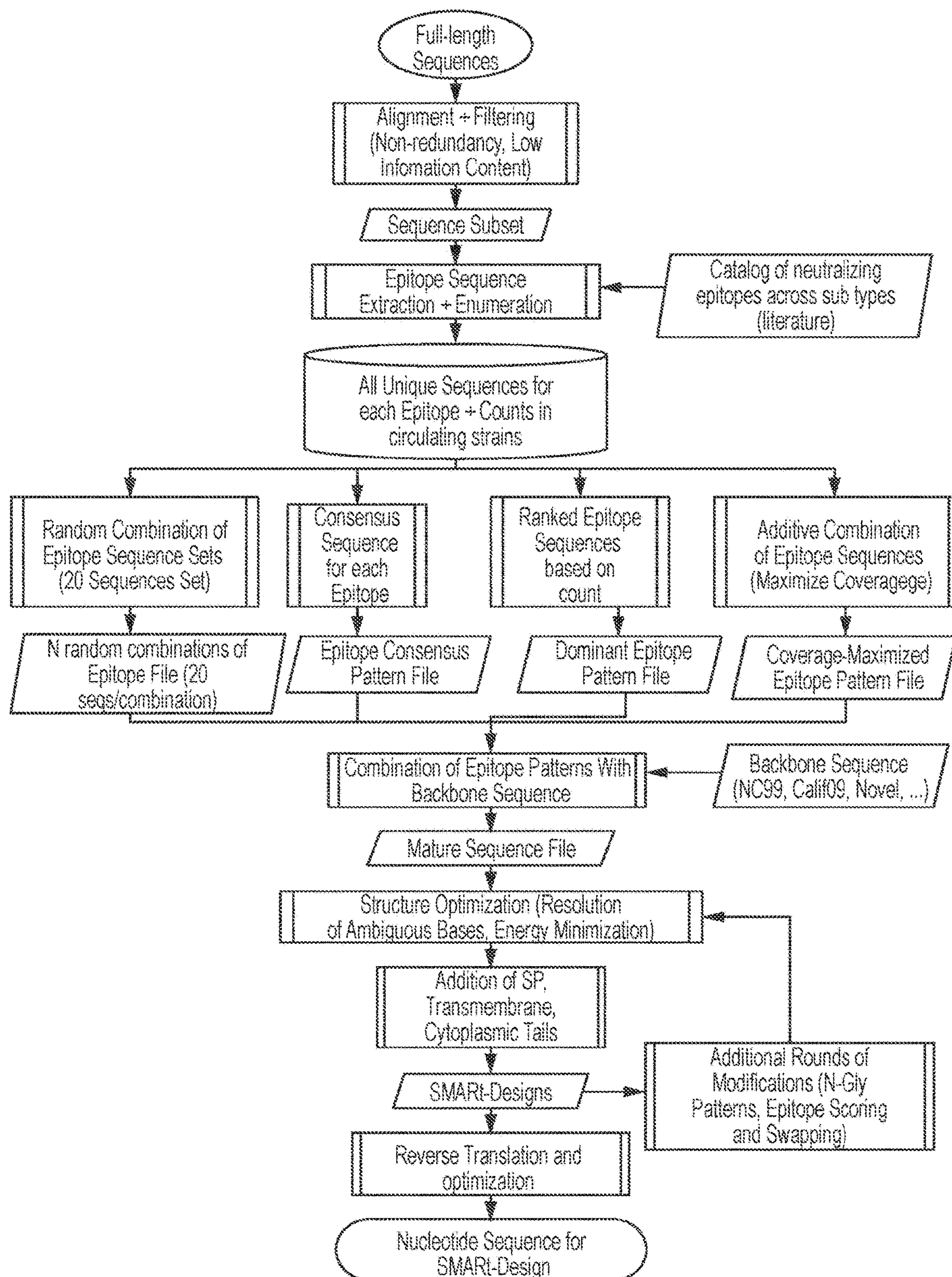


Figure 2

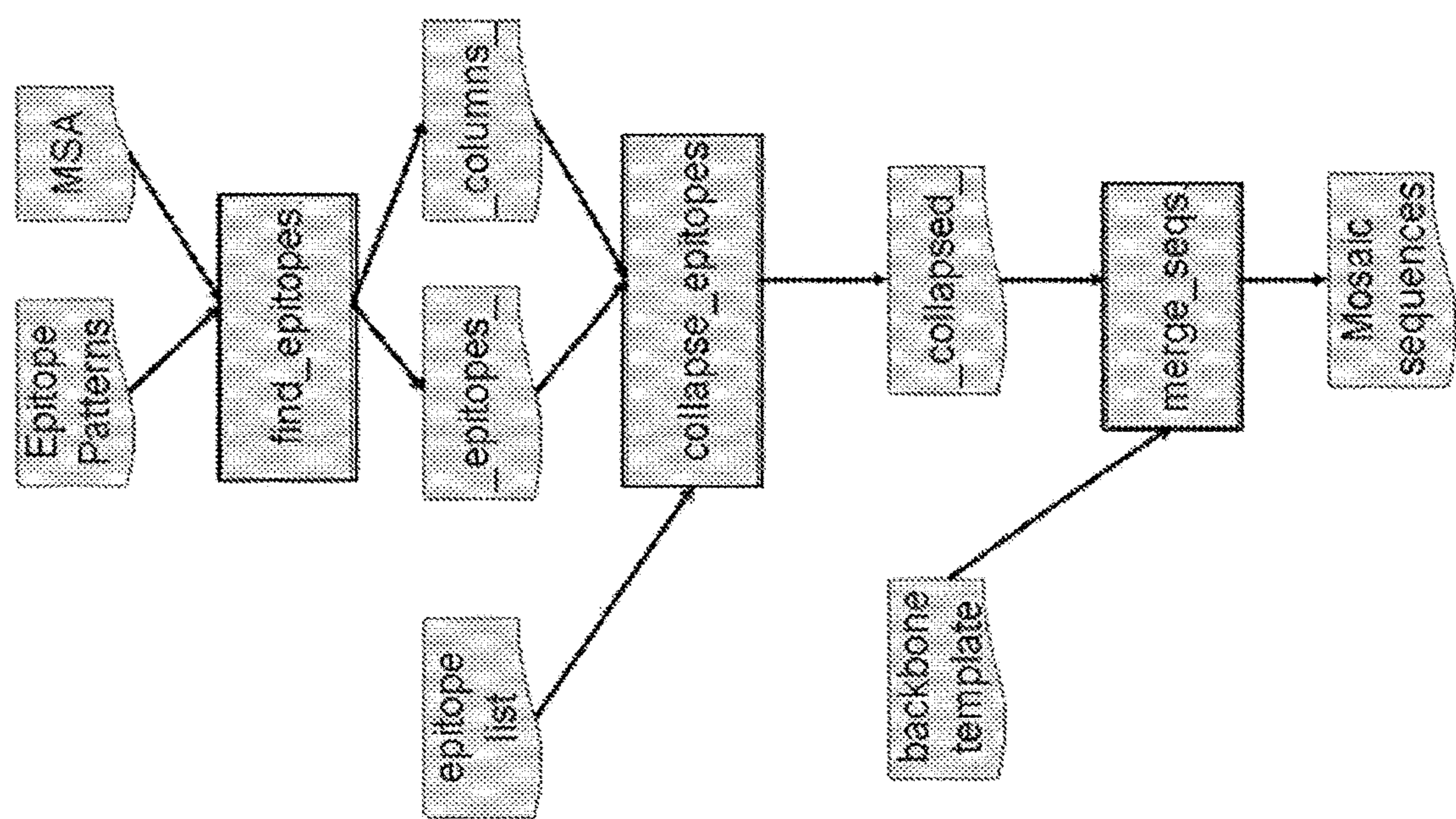


Figure 3

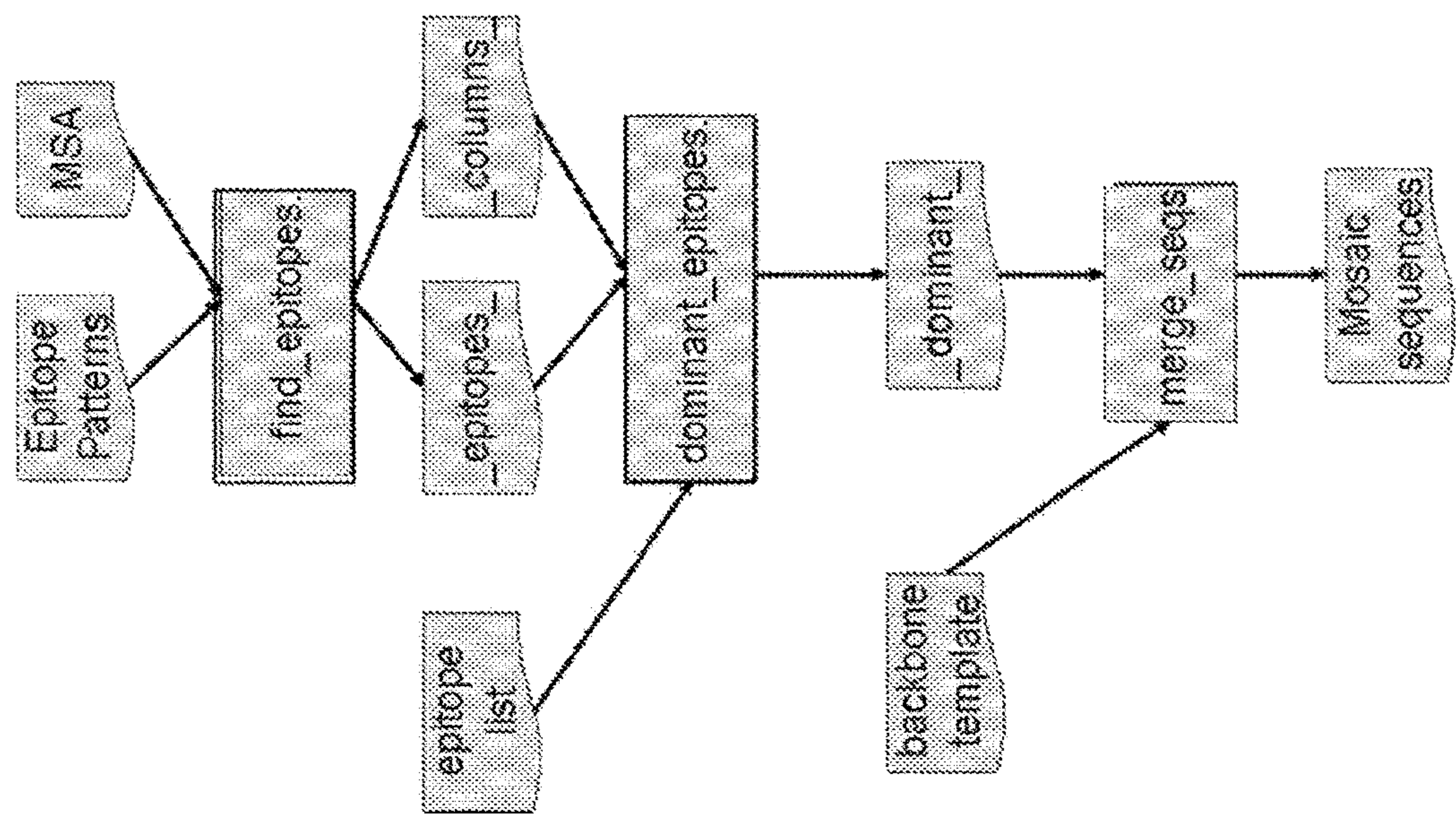


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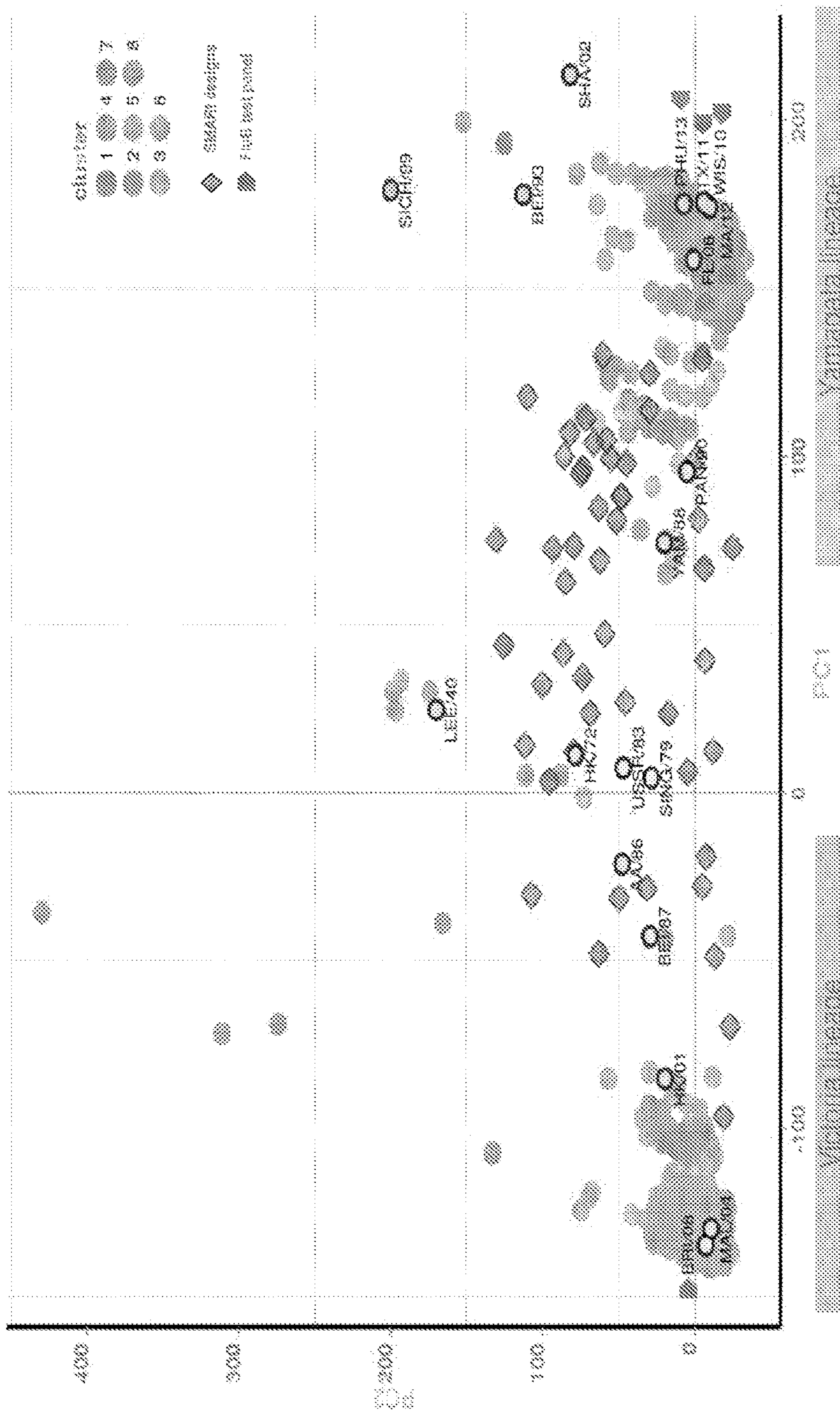


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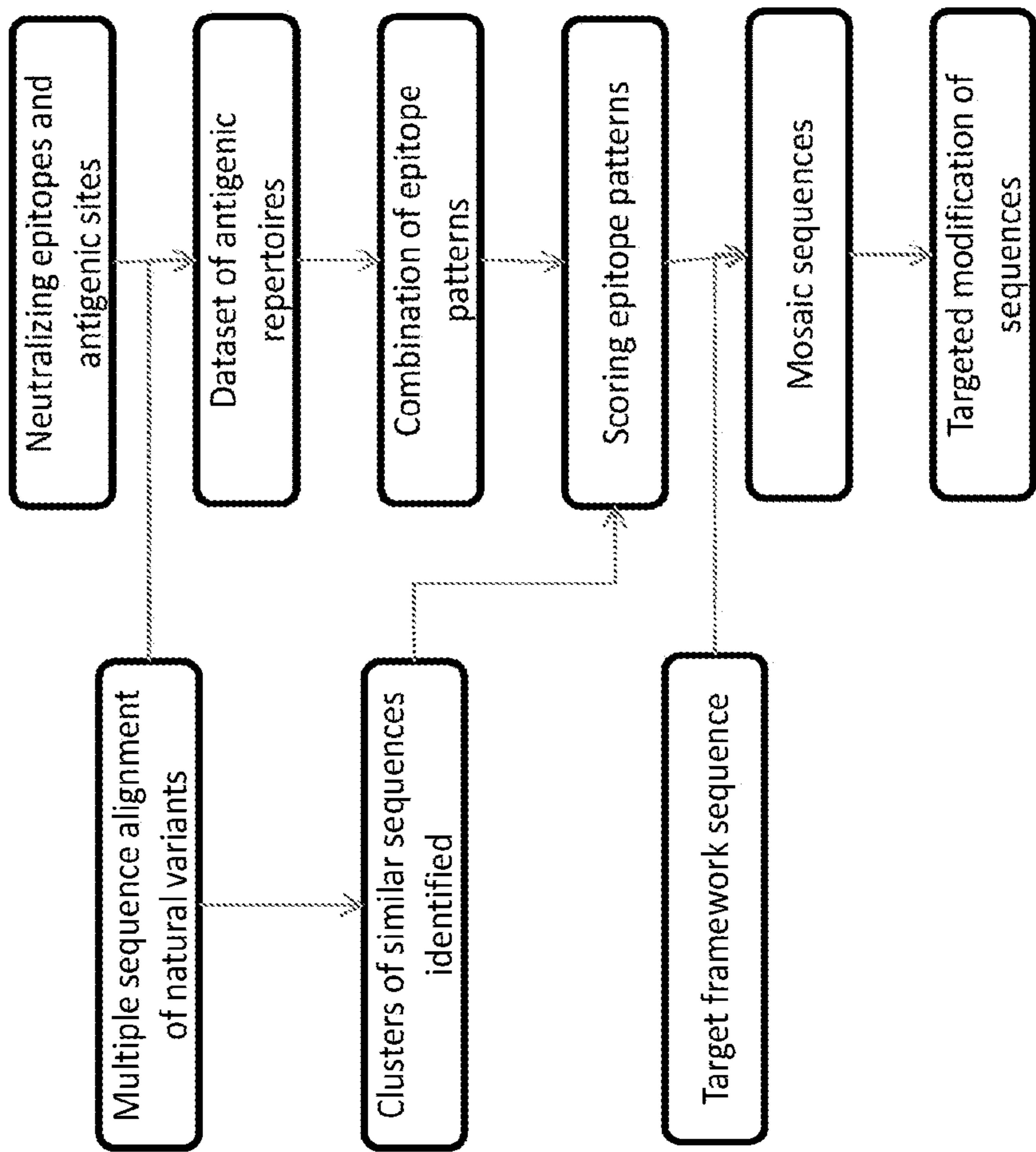


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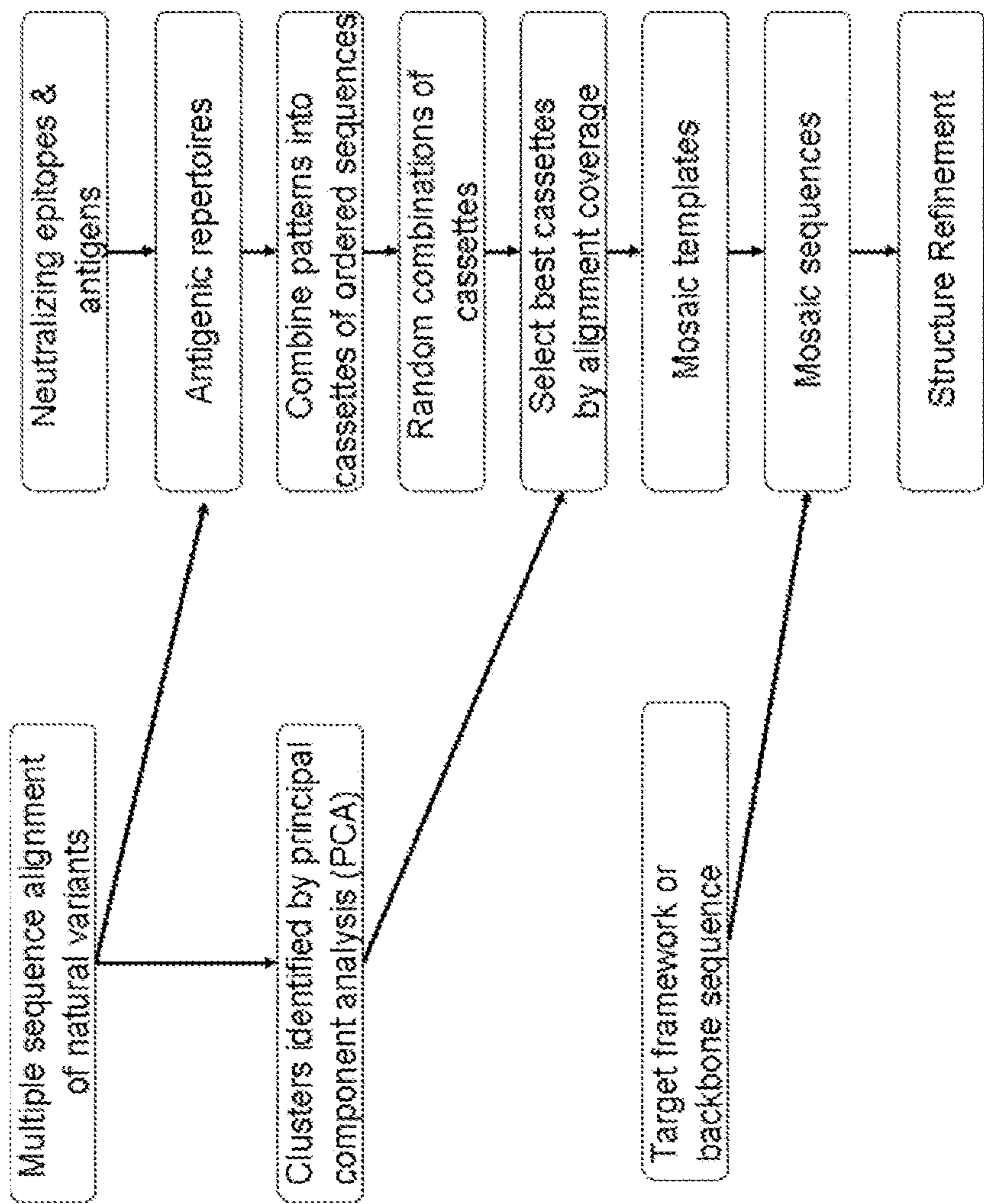


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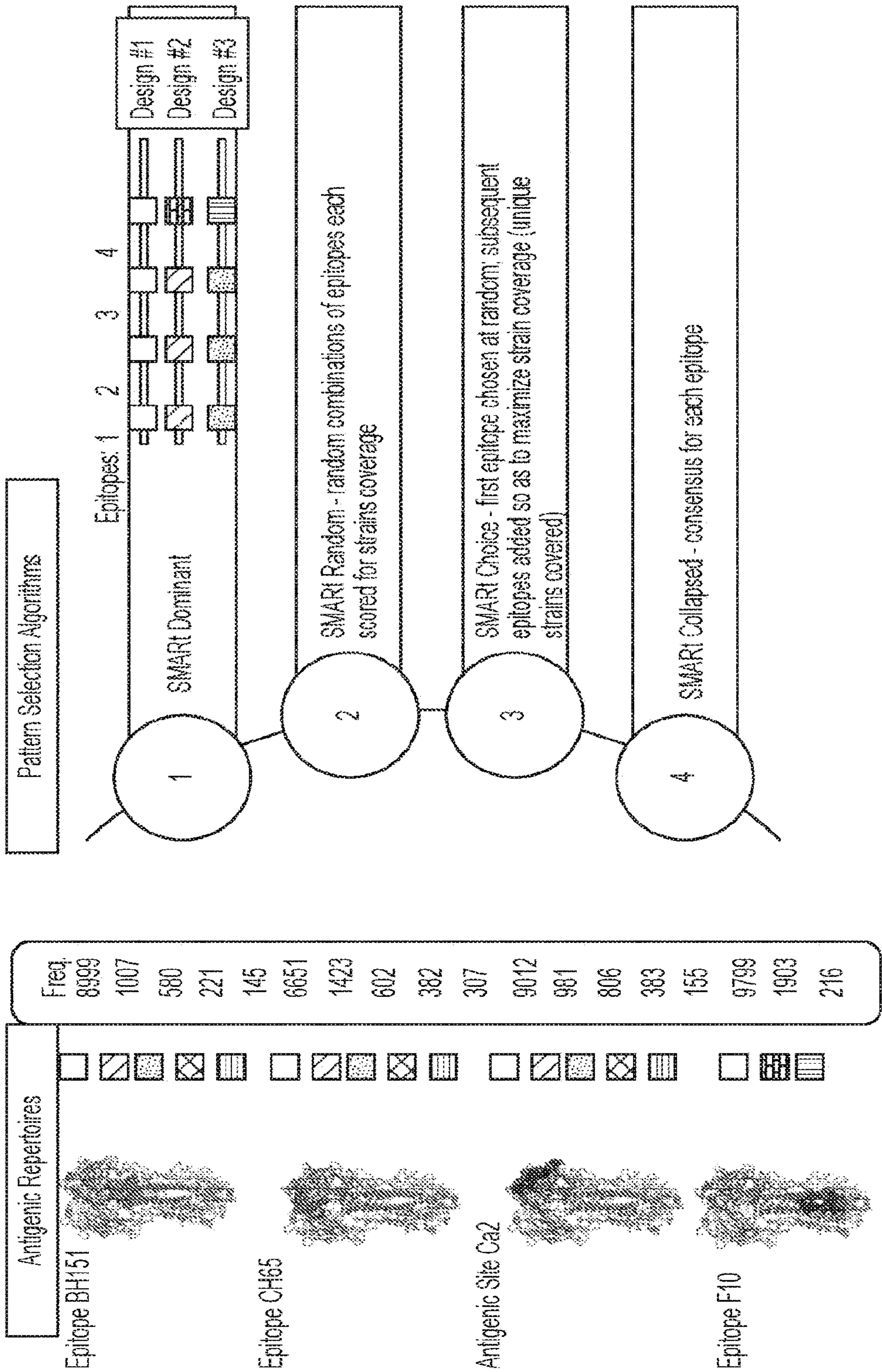


Figure 8

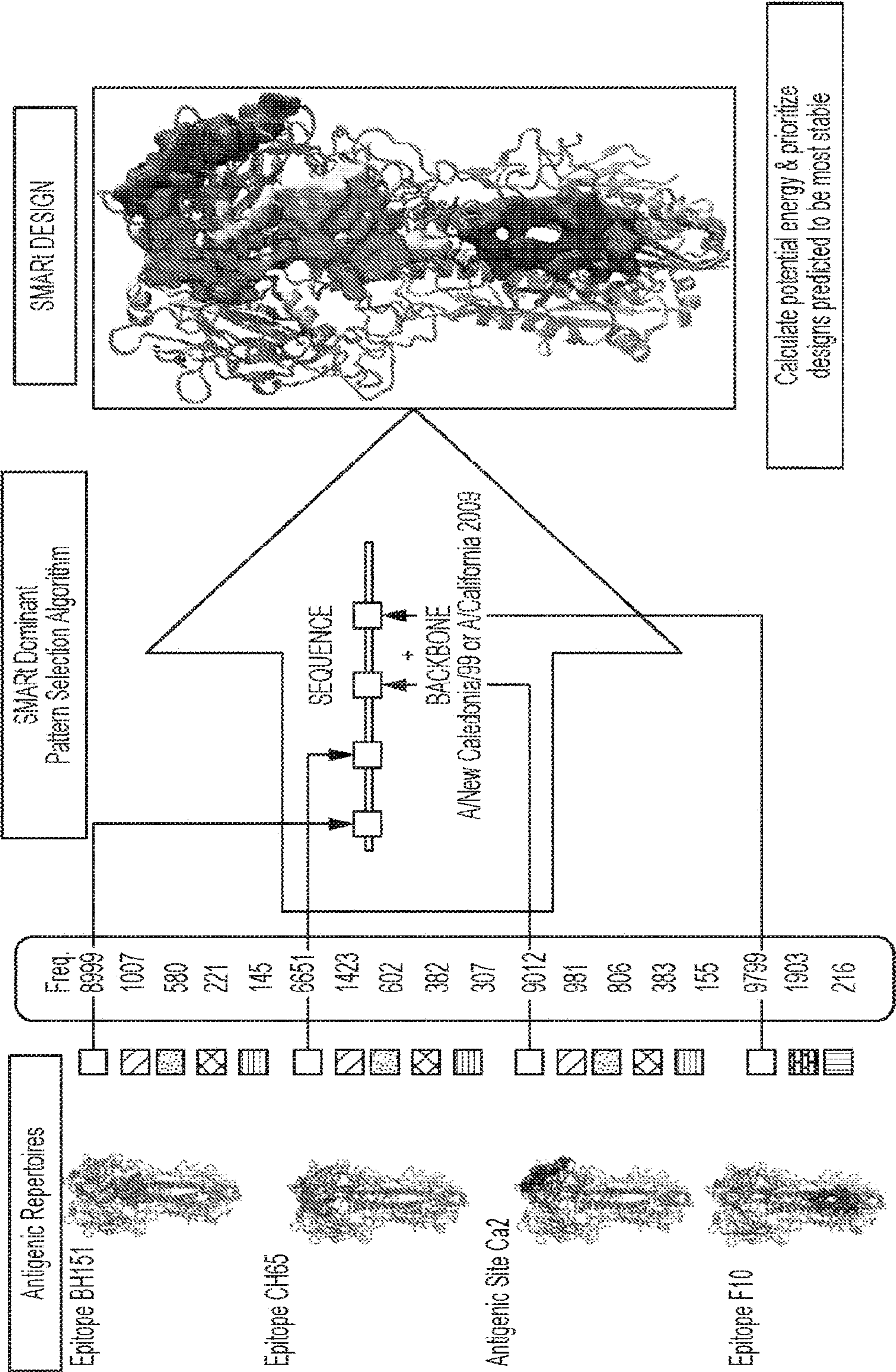


Figure 9

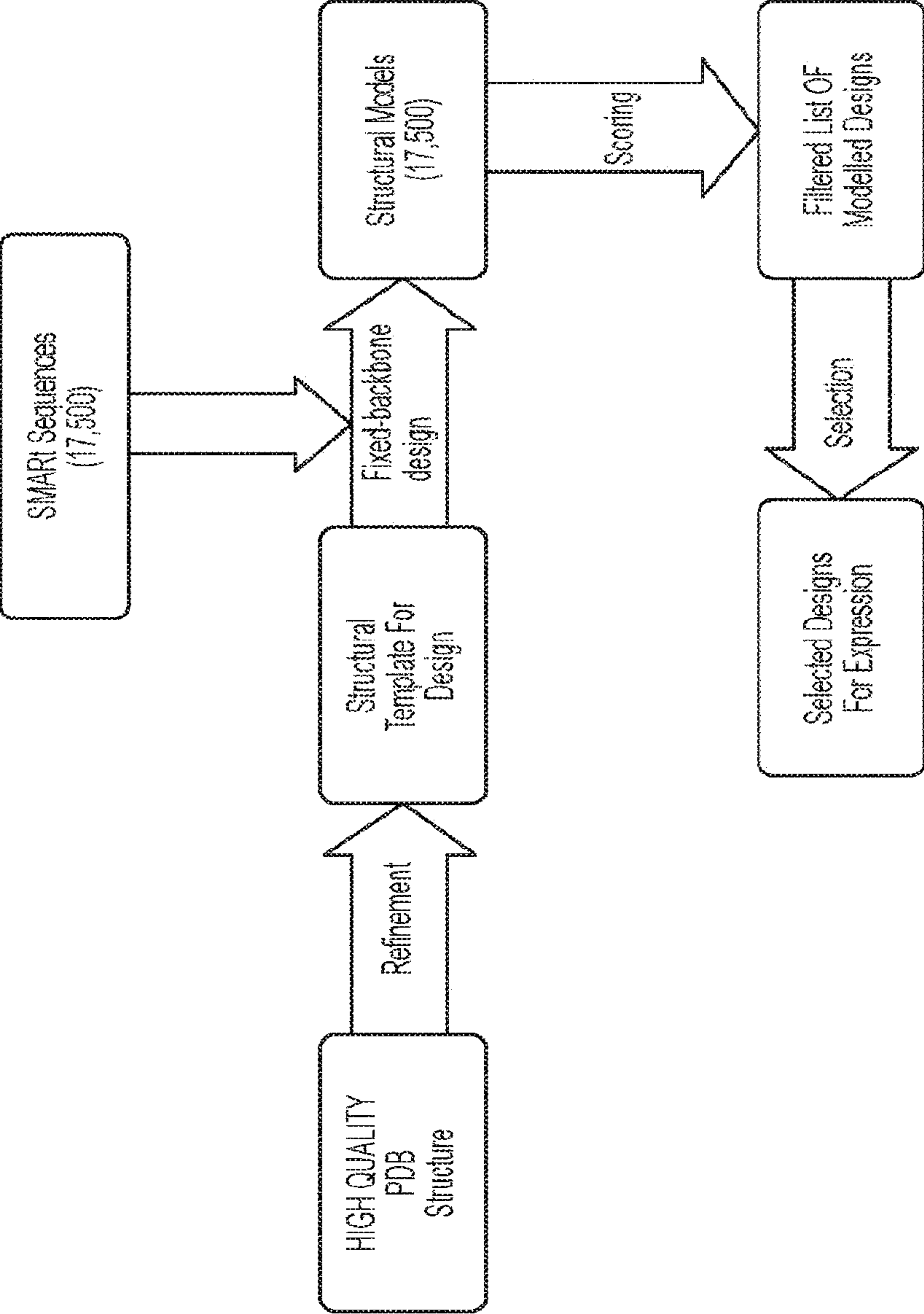


Figure 10

Energy Scoring Function: a set of mathematical equations to compute an "energy" for a given protein conformation. The native conformation is usually the lowest energy conformation

$$\text{Score} = S_{LJ}(\text{atr} + \text{rep}) +$$

$$S_{\text{solvation}} + S_{\text{hb}}(\text{srbb} + \text{trbb} + \text{sc}) +$$

$$S_{\text{dunbrack}} + S_{\text{pair}} - S_{\text{ref}} +$$

$$S_{\text{prob1b}} + S_{\text{intrares}} +$$

$$S_{\text{gb_elec}} + S_{\text{gsolt}} + S_{\text{h2o}}(\text{solv} + \text{hb}) + S_{\text{plane}} +$$

...

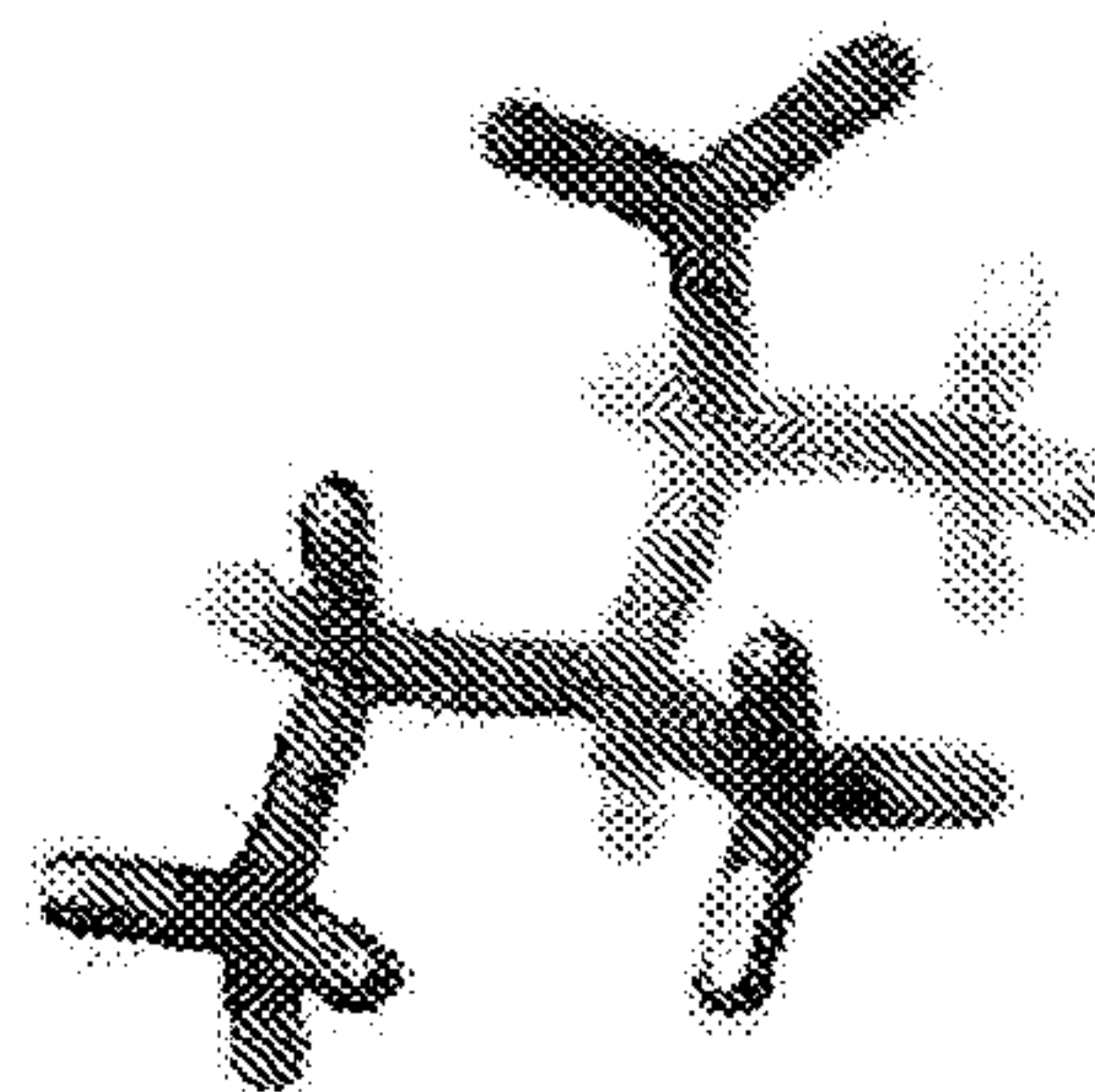


Figure 11

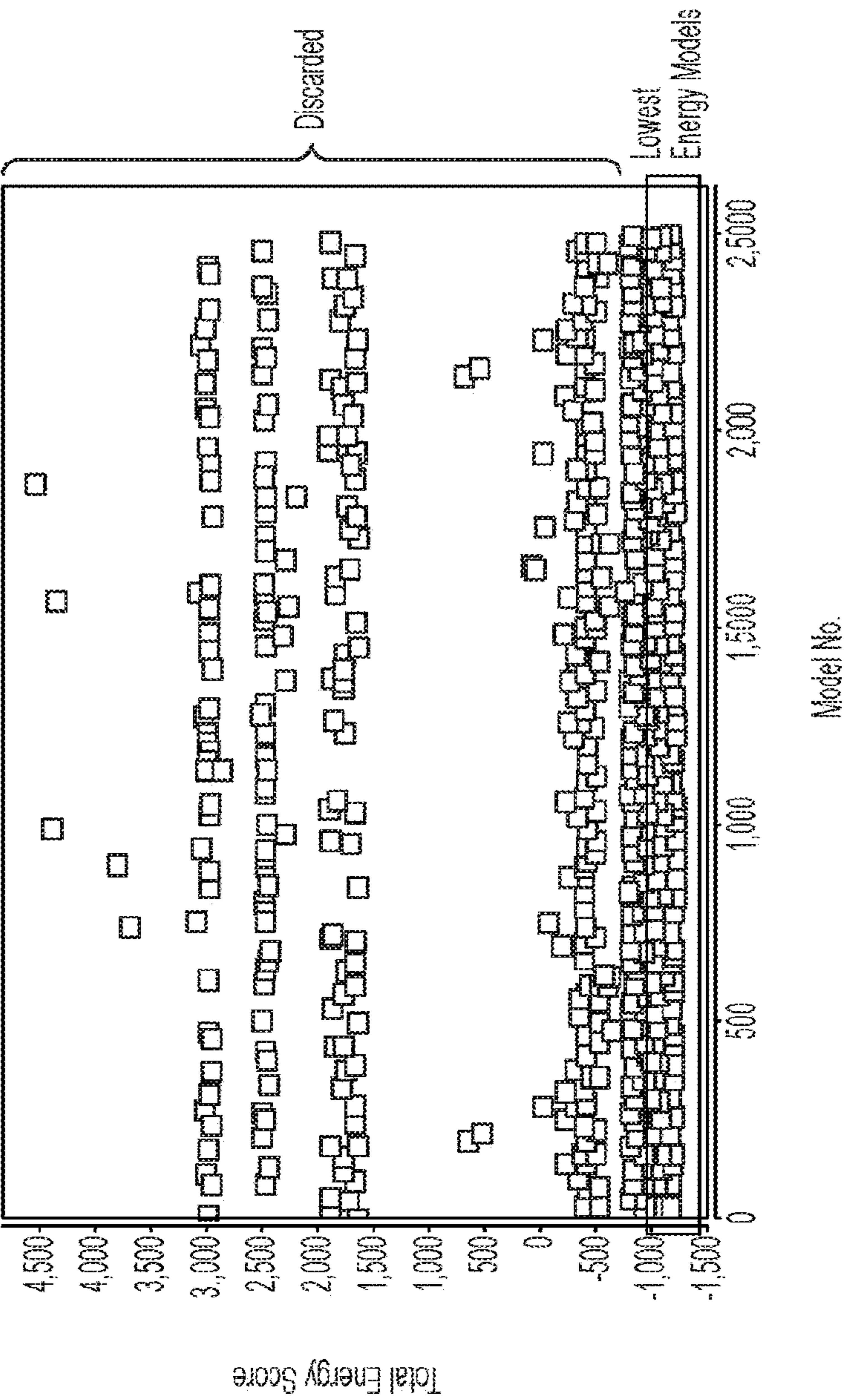


Figure 12

HA	linker	cleavage	Foldon	His
...NITAAS	SGRS	LVPRGS	PGSGYIPEAPRDGGQAYVRKDGENVLLSTFL	GHHHHHH

Figure 13

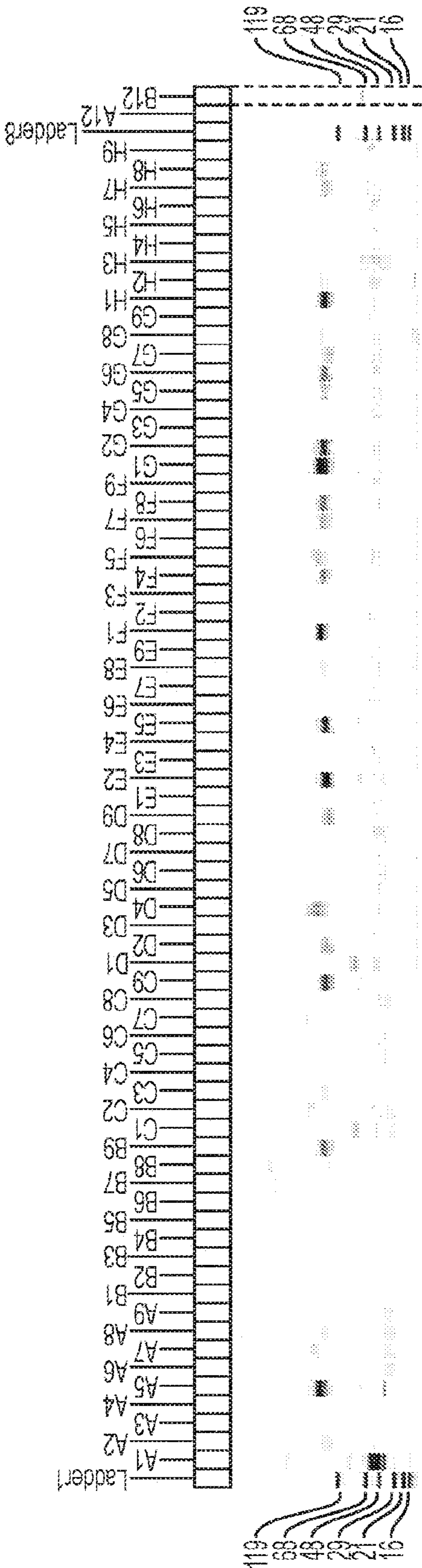


Figure 14

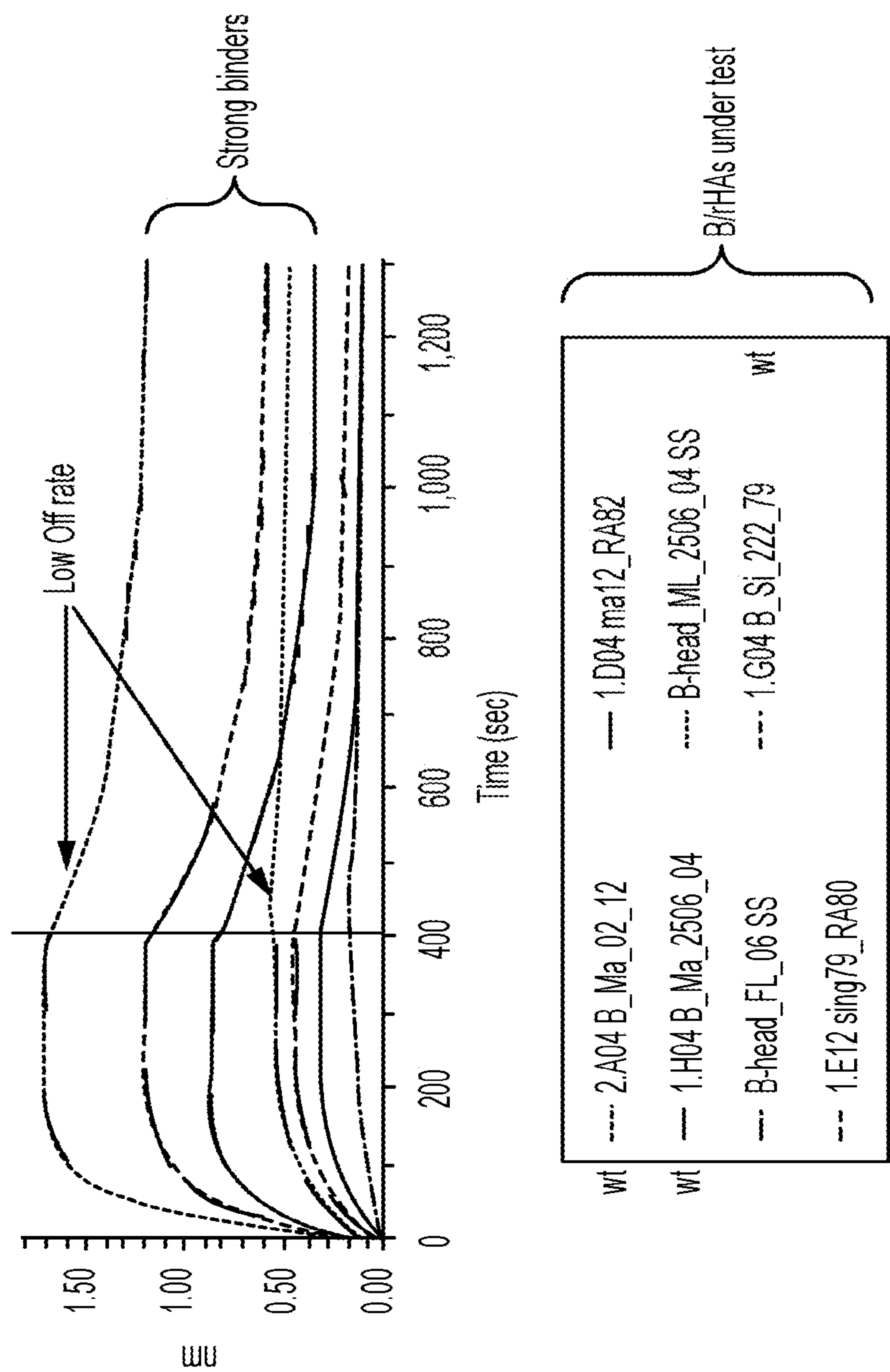


Figure 15

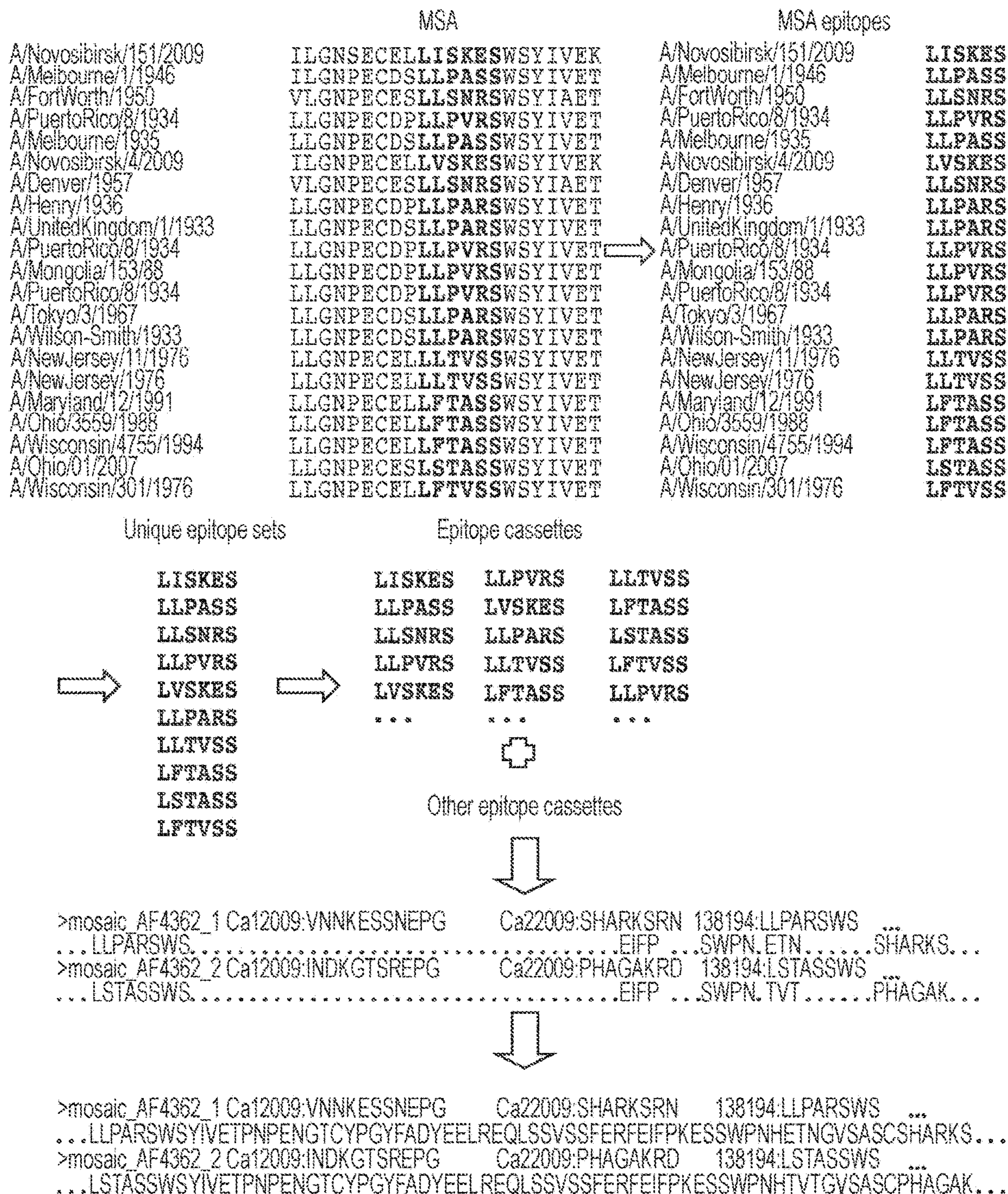


Figure 16

97823 [H32 QDI36 SMP294 T319] [DGW21 K38 TQ42 ID46 T49 VN53 I56]
97824 [H12 H32 VNL36 SLP295 T320] [DGW21 Q38 TQ42 ID46 T49 VN53 I56]
99799 H24 H44 Q46 SM305 VDGW367 K384 TQ386 I391 T395 VN399 I402
joined H...H...QDI...SMP...T...VDGW...K...TQ...ID...T...VN...I

Figure 17A

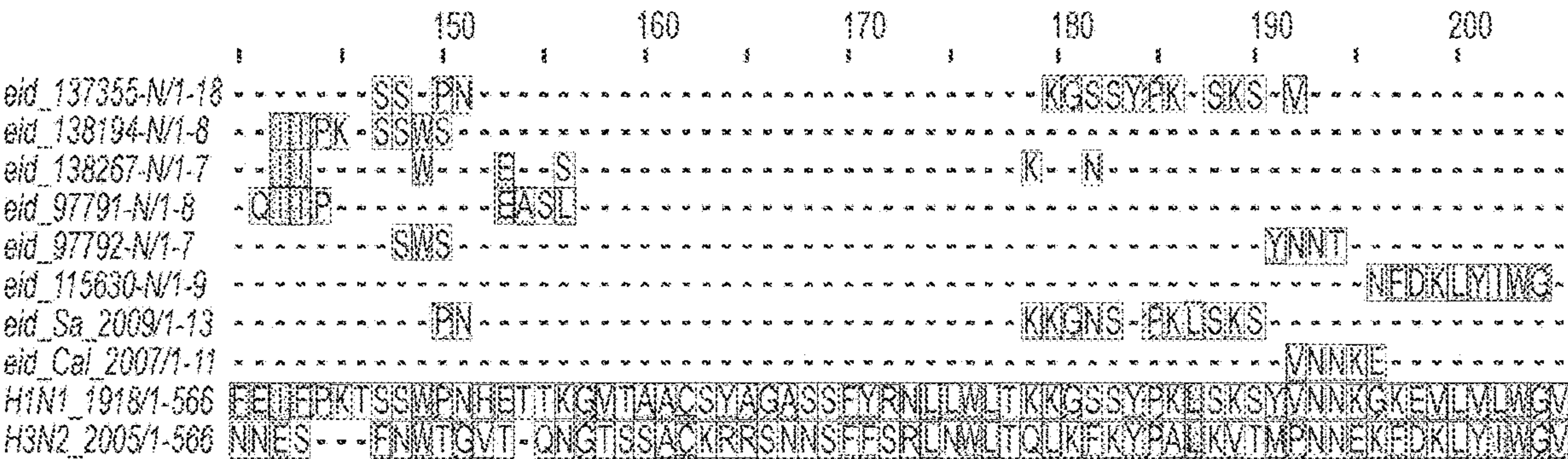


Figure 17B

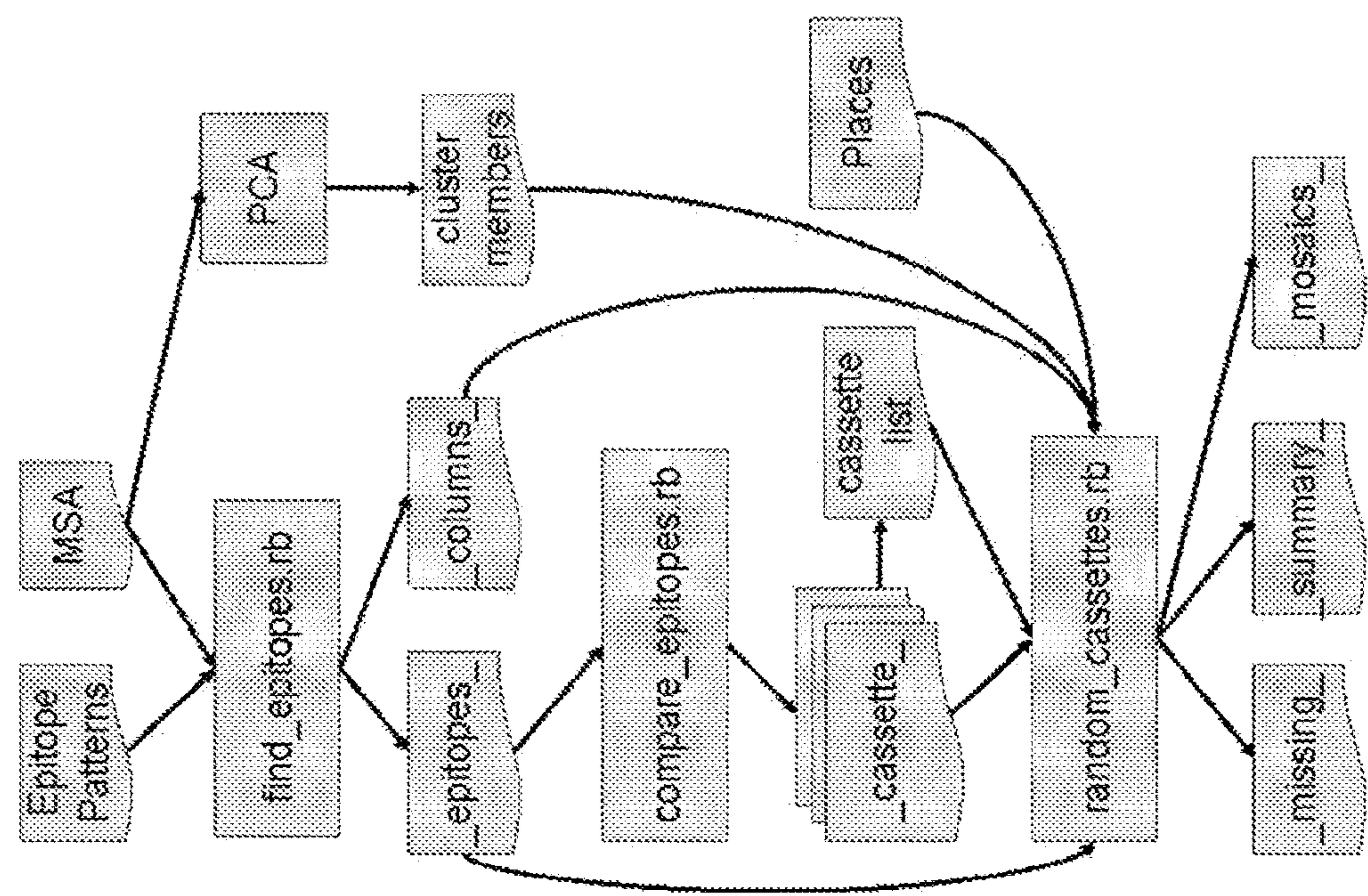


Figure 18

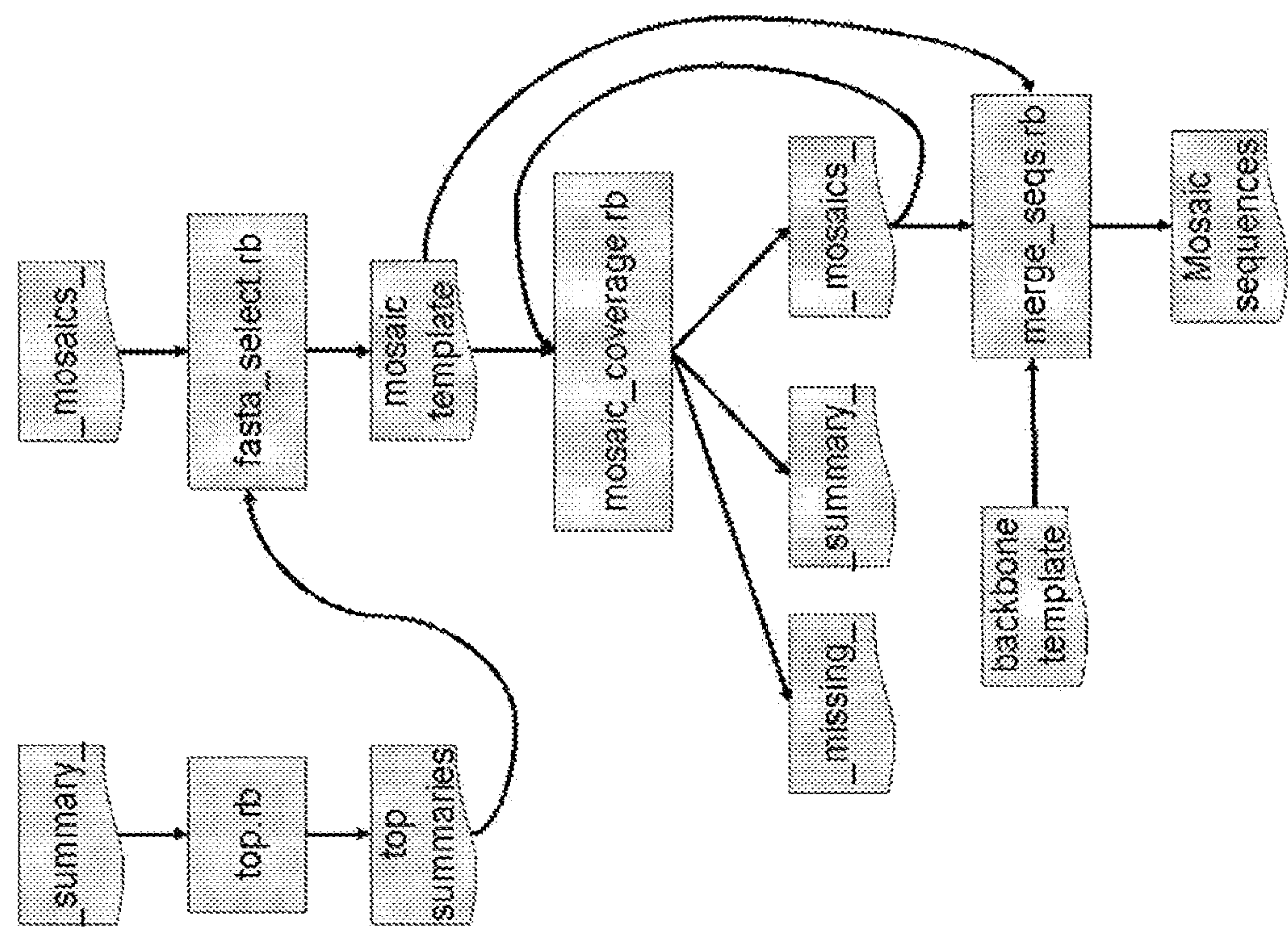


Figure 19

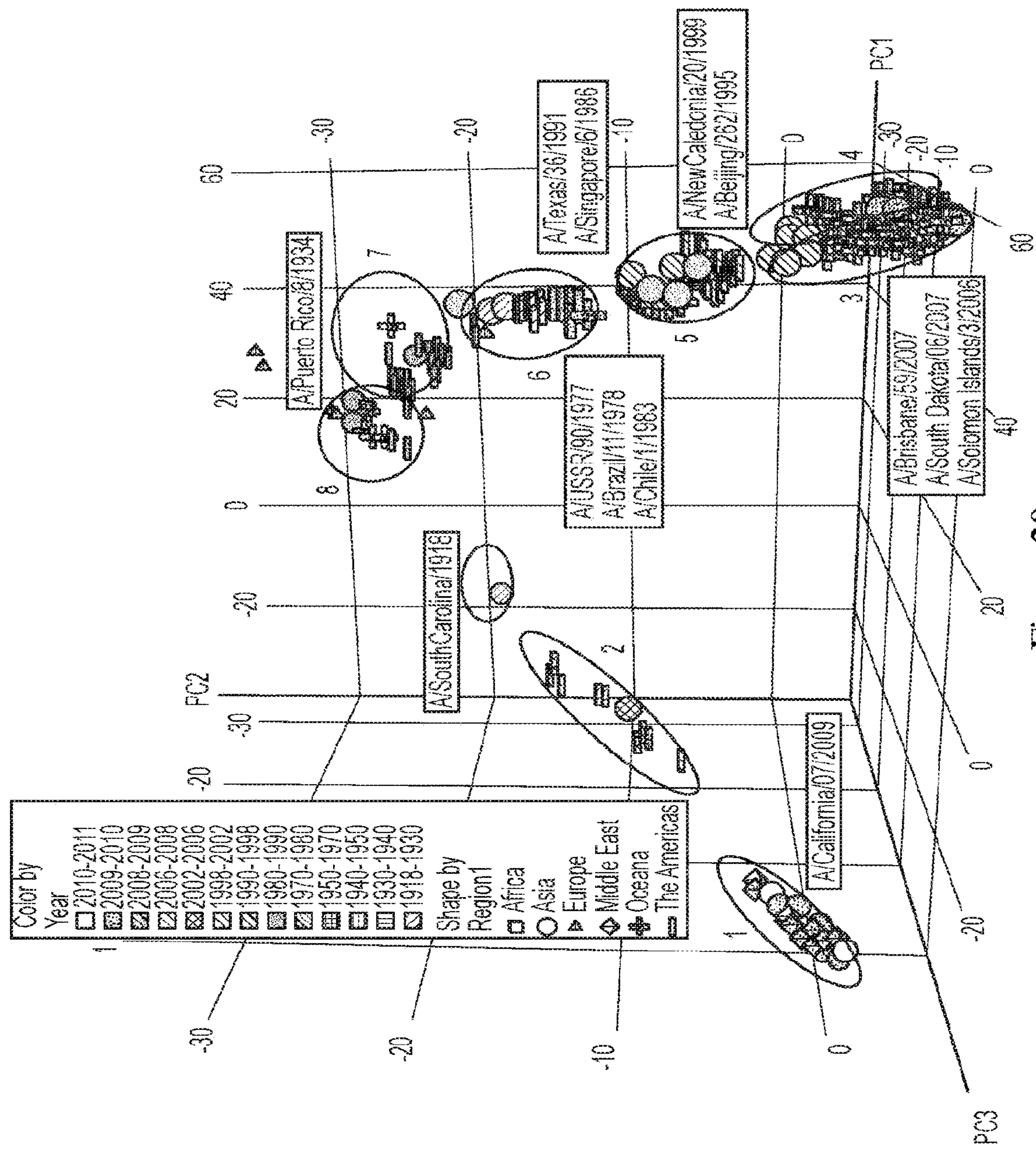


Figure 20

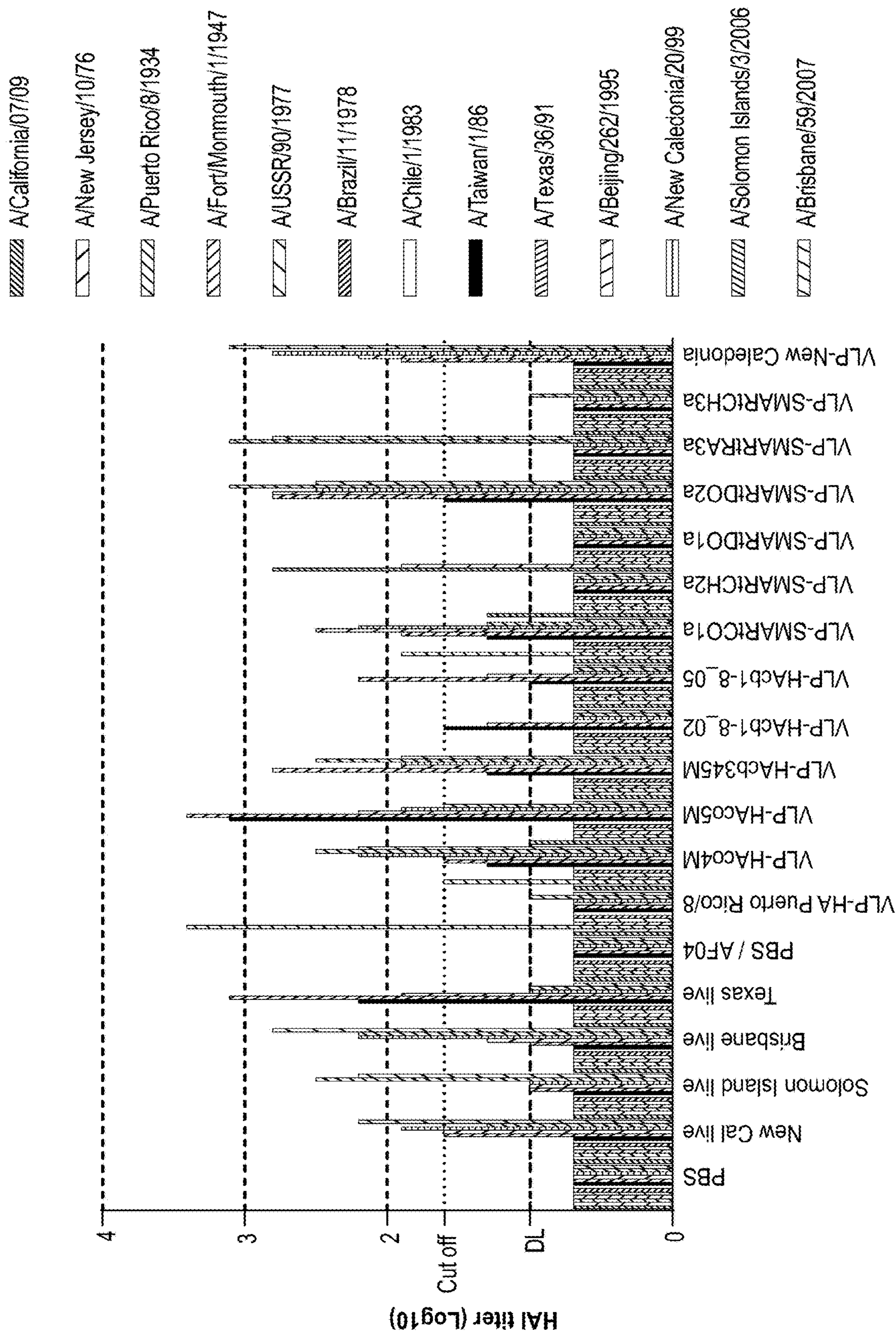


Figure 21

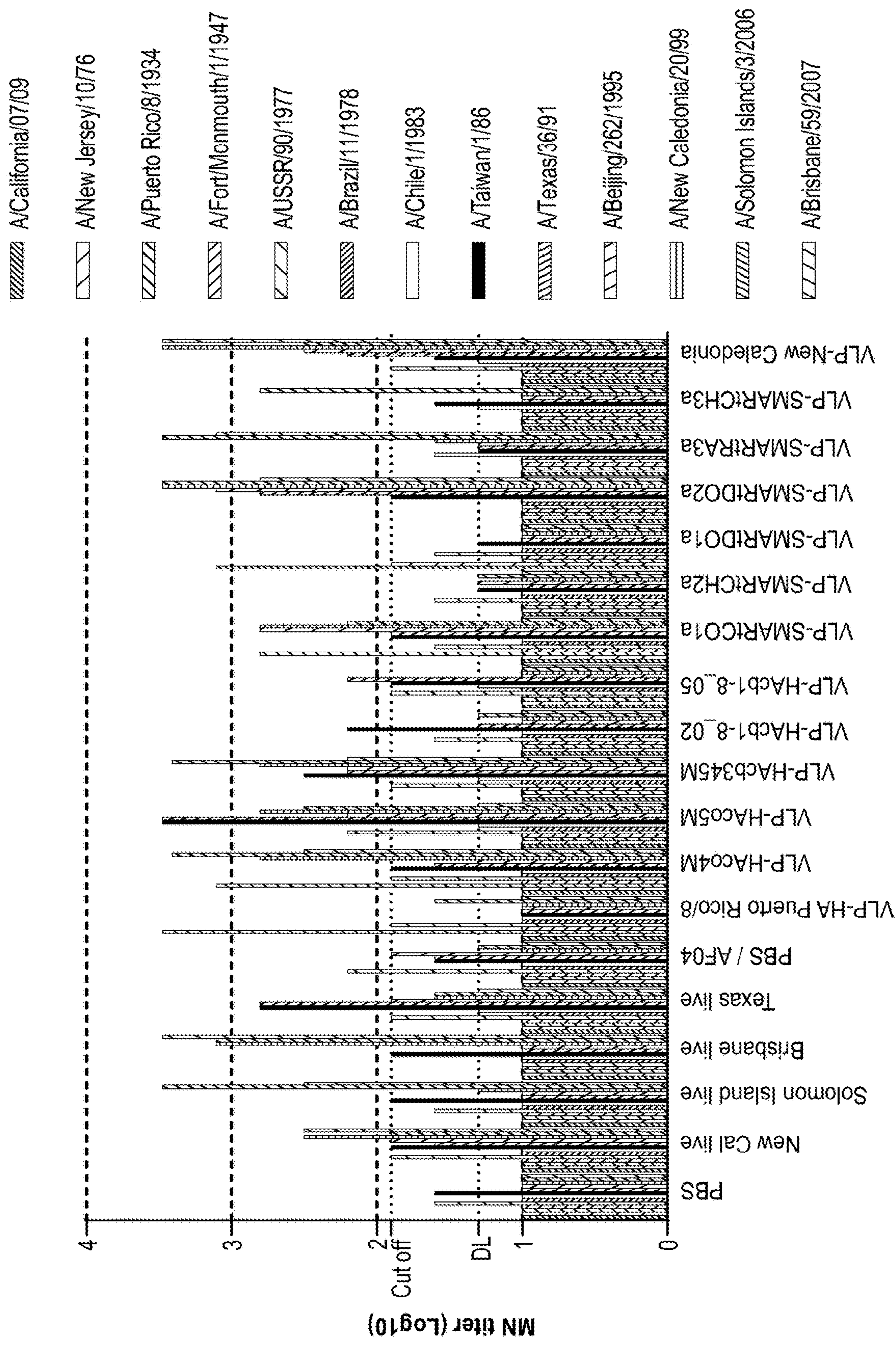


Figure 22

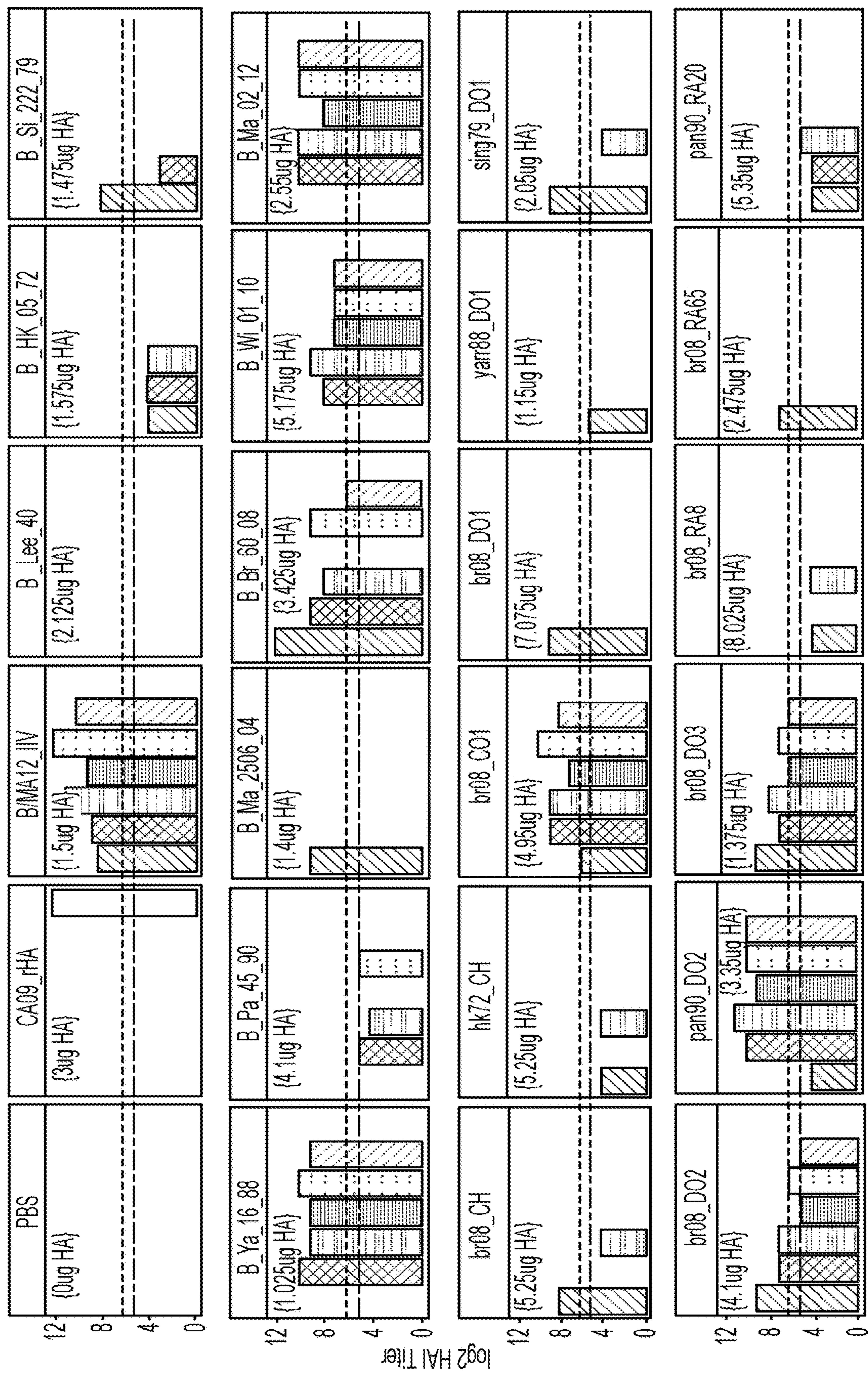


Figure 23

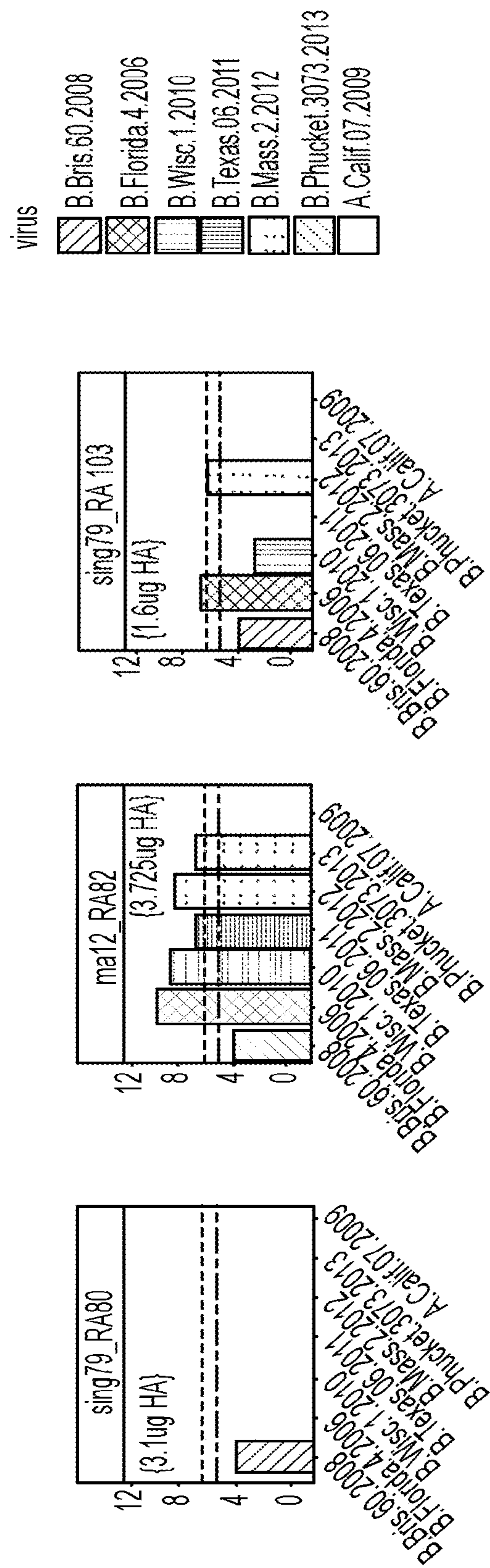


Figure 23
CONTINUED

ENGINEERED INFLUENZA ANTIGENIC POLYPEPTIDES AND IMMUNOGENIC COMPOSITIONS THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional application of U.S. Pat. No. 10,844,097, filed Nov. 30, 2018, which is a 35 U.S.C. § 371 National Stage Application of International Application No. PCT/US17/35747, filed Jun. 2, 2017, which claims priority to U.S. Provisional Patent Application Ser. No. 62/344,862 filed on Jun. 2, 2016, the entirety of each of which is hereby incorporated by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Jan. 21, 2021, is named SPR-010US2_SL.txt and is 143,050 bytes in size.

BACKGROUND

Influenza has a long standing history of pandemics, epidemics, resurgences and outbreaks. Vaccines have been the most effective defense against influenza. However, the effort to design and manufacture vaccines that induce strain-specific immunity year-over-year has been difficult and influenza continues to cause significant health problems across the globe. Indeed, currently marketed influenza vaccines must be updated annually based on predicted strains that will be present in human populations in the impending season.

Current influenza vaccines are based on inducing immunity to the hemagglutinin antigen present on the surface of influenza viruses. Hemagglutinin (HA) is a glycoprotein responsible for the binding of the influenza virus to cells with sialic acid-containing on surface structures on their membranes, and is highly variable across influenza virus strains. Among the current strategies for vaccination against influenza, the development of a universal vaccine holds the promise to increase the breadth of current strain-specific vaccines by focusing on relatively conserved regions of HA.

SUMMARY

The present invention provides, among other things, a novel and improved method for generating “mosaic” influenza antigenic polypeptides including hemagglutinin (HA) and neuraminidase (NA) polypeptides based on unique combination of epitope patterns that maximize exposure to epitopes present across multiple HA or NA sequences and therefore improve influenza strain coverage. In particular, the present invention provides engineered influenza B polypeptides that provide for improved protective immunity (e.g., a broad reactive immune response) to multiple influenza B virus isolates. The engineered HA polypeptides were developed by using a unique combination of epitope patterns to create “mosaic” HA polypeptides that maximize exposure to epitopes present across multiple HA sequences and therefore improved influenza strain coverage.

In one aspect, the present invention provides a method of engineering a mosaic influenza hemagglutinin (HA) polypeptide, comprising obtaining HA amino acid sequences from multiple circulating strains of a particular type and/or

subtype of influenza virus; aligning the HA amino acid sequences to generate an alignment; identifying the positions of amino acids comprising known epitopes and antigenic regions; compiling the amino acid residues across the alignment at the identified positions for each epitope and antigenic region; defining a set of amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino acid sequence pattern in the set is represented only once; selecting a sequence from the set for each epitope or antigenic region; and inserting one or more selected sequences into corresponding locations in a structural backbone of HA to generate a mosaic influenza HA polypeptide.

As used herein, “corresponding locations”, in the context of an influenza HA or NA polypeptide sequence, generally refer to the locations that correspond to the location of the known epitopes and antigenic regions. Typically, “corresponding locations” on a polypeptide of interest (e.g., an HA polypeptide) are designated using a canonical numbering system based on a related reference polypeptide. Residues at “corresponding locations” of different HA or NA polypeptides need not actually be at the same locations.

In another aspect, the present invention provides a method of engineering a mosaic influenza hemagglutinin (HA) polypeptide, comprising obtaining HA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus; aligning the HA amino acid sequences to generate an alignment; identifying the positions of amino acids comprising known epitopes and antigenic regions across the alignment; compiling the amino acid residues at the identified positions for each epitope and antigenic region; defining a set amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino sequence pattern in the set is represented only once; generating a consensus sequence from each set for each epitope or antigenic region; and inserting one or more consensus sequences into corresponding locations in a structural backbone of HA to generate a mosaic influenza HA polypeptide.

In some embodiments, the methods described herein may be performed computationally. In particular embodiments, the methods may be performed using an algorithm.

In some embodiments, the locations in the structural model do not overlap.

In some embodiments, the steps of various methods according to the present invention are performed in silico by a suitably programmed computer system.

In some embodiments, a method of the present invention further comprises measuring the structural stability of the mosaic influenza HA polypeptide.

In some embodiments, measuring the stability comprises calculating the folding energy of each mosaic influenza HA polypeptide and selecting polypeptides that are likely to fold into a native-like conformation.

In some embodiments, the selecting step used in a method of the present invention further comprises ranking the selected sequences by sequence identity, geographical location and/or isolation date of the type or subtype of influenza virus. In some embodiments, the selecting step is random. In some embodiments, the sequences are selected so the mosaic influenza HA polypeptide elicits a broadly neutralizing immune response against multiple circulating influenza strains. In some embodiments, the sequences are selected according to a pre-determined algorithm.

In some embodiments, prior to the selecting step, the patterns in the set are weighted by their frequency of occurrence. In some embodiments, the selecting step com-

prises selecting the most frequently occurring sequence for each of the epitopes or antigenic regions. In some embodiments, the selecting step comprises selecting the second most frequently occurring sequence for each of the epitopes or antigenic regions. In some embodiments, the selecting step comprises selecting the third most frequently occurring sequence for each of the epitopes or antigenic regions.

In some embodiments, the obtaining step further comprises identifying redundant sequences by screening the HA amino acid sequences for those with 100%, or greater than 99.9%, 99% 98%, 97%, or 96% sequence identity, sequence identity, and removing all but one of the redundant sequences.

In some embodiments, the obtaining step according to a method of the present invention further comprises identifying and removing redundant sequences.

In some embodiments, the obtaining step comprises obtaining 100-5000 HA amino acid sequences from multiple circulating influenza strains.

In some embodiments, the obtaining step comprises obtaining all publicly available HA amino acid sequences.

In some embodiments, a method according to the present invention further comprises: generating a nucleic acid sequence corresponding to the mosaic influenza HA polypeptide; cloning the nucleic acid sequence into a mammalian expression vector; and transfecting a mammalian host cell with the mammalian expression vector. In some embodiments, the mammalian host cell is a Vero cell.

In some embodiments, the epitopes are neutralizing epitopes. In some embodiments, the epitopes are discontinuous epitopes. In some embodiments, the epitopes are continuous epitopes. In some embodiments, the epitopes are B cell epitopes.

In some embodiments, a method according to the present invention involves obtaining HA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus which is a type A influenza virus. In some embodiments, the influenza A virus is selected from subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, or H16. In some embodiments, the influenza virus is elected from the group consisting of H1N1, H3N2, H5N1, and H7N9. In some embodiments, the type A virus is a seasonal strain. In particular embodiments, a method according to the present invention involves obtaining HA amino acid sequences from multiple circulating strains including/Texas/36/1991, A/Singapore/1986, A/New Caledonia/20/1999, A/Solomon Islands/03/2006, and A/Brisbane/59/2007 and A/Wisconsin/67/2005. In some embodiments, the type A virus is a pandemic strain. In some embodiments, a method according to the present invention involves obtaining HA amino acid sequences from multiple circulating strains including A/California/07/2009, A/California/04/2009, A/Belgium/145/2009, A/South Carolina/01/1918 and A/New Jersey/1976.

In some embodiments, a method according to the present invention involves obtaining HA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus is a type B influenza virus. In some embodiments, the influenza B virus is a Yamagata lineage strain. In some embodiments, the influenza B virus is a Victoria lineage strain. In some embodiments, the influenza B virus strain circulated prior to the split into distinct lineages and is therefore neither a Yamagata nor Victoria lineage. In some embodiments, a method according to the present invention involves obtaining HA amino acid sequences from the influenza B virus selected from B/Hong Kong/330/2001, B/Hong Kong/05/1972, B/Lee/40, B/Mas-

sachusetts/02/2012, B/Panama/45/1990, B/Singapore/222/79, B/Victoria/02/1987, B/Yamagata/16/1988, or B/Brisbane/60/2008.

In yet another aspect, the present invention provides a method of engineering a mosaic influenza neuraminidase (NA) polypeptide, comprising: obtaining NA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus; aligning the NA amino acid sequences to generate an alignment; identifying the positions of amino acids comprising known epitopes and antigenic regions; compiling the amino acid residues across the alignment at the identified positions for each epitope and antigenic region; defining a set of amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino sequence pattern in the set is represented only once; selecting a sequence from the set for each epitope or antigenic region; and inserting selected sequences into corresponding locations in a structural backbone of NA to generate a mosaic influenza NA polypeptide.

In still another aspect, the present invention provides a method of engineering a mosaic influenza neuraminidase (NA) polypeptide, comprising: obtaining NA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus; aligning the NA amino acid sequences to generate an alignment; identifying the positions of amino acids comprising known epitopes and antigenic regions across the alignment; compiling the amino acid residues at the identified positions for each epitope and antigenic region; defining a set amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino sequence pattern in the set is represented only once; generating a consensus sequence from each set for each epitope or antigenic region; and inserting the consensus sequences into corresponding locations in a structural backbone of NA to generate a mosaic influenza NA polypeptide.

In some embodiments, the locations in the structural model do not overlap.

In some embodiments, the steps according to various methods described herein are performed in silico by a suitably programmed computer system.

In some embodiments, a method according to the present invention further comprises measuring the stability of the mosaic influenza NA polypeptide. In some embodiments, measuring the stability comprises calculating the folding energy of each mosaic influenza NA polypeptide and selecting polypeptides that are likely to fold into a native-like conformation.

In some embodiments, the selecting step further comprises ranking the selected sequences by sequence identity, geographical location and/or isolation date of the type or subtype of influenza virus. In some embodiments, the selecting step is random. In some embodiments, the sequences are selected so the mosaic influenza NA polypeptide elicits a broadly neutralizing immune response against the multiple circulating influenza strains. In some embodiments, the sequences are selected according to a pre-determined algorithm.

In some embodiments, prior to the selecting step, the patterns in the set are weighted by their frequency of occurrence. In some embodiments, the selecting step comprises selecting the most frequently occurring sequence for each of the epitopes or antigenic regions. In some embodiments, the selecting step comprises selecting the second most frequently occurring sequence for each of the epitopes or antigenic regions. In some embodiments, the selecting

5

step comprises selecting the third most frequently occurring sequence for each of the epitopes or antigenic regions.

In some embodiments, the obtaining step according to a method of the present invention further comprises identifying redundant sequences by screening the NA amino acid sequences for those with 100%, or greater than 99.9%, 99% 98%, 97%, or 96% sequence identity, and removing all but one of the redundant sequences.

In some embodiments, the obtaining step according to a method of the present invention further comprises identifying and removing redundant sequences.

In some embodiments, the obtaining step comprises obtaining 100-5000 NA amino acid sequences from multiple circulating influenza strains.

In some embodiments, the obtaining step comprises obtaining all publicly available NA amino acid sequences.

In some embodiments, a method of the present invention further comprises generating a nucleic acid sequence corresponding to the mosaic influenza HA polypeptide; cloning the nucleic acid sequence into a mammalian expression vector; and transfecting a mammalian host cell with the mammalian expression vector. In some embodiments, the mammalian host cell is a Vero cell.

In some embodiments, the epitopes are neutralizing epitopes. In some embodiments, the epitopes are discontinuous epitopes.

Among other things, the present invention provides an engineered mosaic influenza HA or NA polypeptide according to various methods described here in, or a combination thereof.

In some embodiments, the present invention provides an engineered mosaic influenza hemagglutinin (HA) polypeptide comprising a mosaic amino acid sequence pattern defined by amino acid substitutions at residues 110, 140, 141, 143, 145, 146, 153, 154, 155, 156, 165, 174, 175, 176, 177, 179, 180, 181, 209, 210, 211, 212, 215, 216, 217, 228, 258, 259, 279 or a subset thereof, in the receptor binding site (RBS), as indexed by reference to a B/Brisbane/60/2008 amino acid sequence.

In some embodiments, the present invention provides an engineered mosaic influenza hemagglutinin (HA) polypeptide comprising a mosaic amino acid sequence pattern defined by amino acid substitutions 110F, 140I, 141D/N, 143E, 145A, 146P, 153G, 154T, 155S, 156G/R, 165S/I/N, 174A, 175V, 176P, 177K, 179D, 180N/S, 181N, 209H, 210S, 211D, 212N/D, 215Q, 216M, 217K/V, 228F, 258S, 259G, 279Q, or a subset thereof in the receptor binding site (RBS) as indexed by reference to a B/Brisbane/60/2008 amino acid sequence.

In some embodiments, the subset comprises at least 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues. In some embodiments, the subset comprises at least 3, 4, 5, 6, 7, 8, 9, or 10 of the referenced residues.

In some embodiments, an engineered mosaic influenza HA polypeptide according to the present invention comprises a backbone from type B influenza virus. In some embodiments, the type B influenza virus is a Yamagata lineage strain. In some embodiments, the type B influenza virus is a Victoria lineage strain. In some embodiments, the type B influenza virus is selected from CAA25425|HA|Human|fluB|B/Singapore/222/79|Singapore|1979|, AGL06036|HA|Human|fluB|B/Massachusetts/02/2012|USA|2012/03/13|, ABL76694|HA|Human|fluB|B/Panama/45/1990|Panama|1990/03/07|, or AFH57909|HA|Human|fluB|B/Brisbane/60/2008|Australia|2008|.

6

In some embodiments, the present invention provides an engineered mosaic influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 40, wherein the amino acid sequence is not a naturally-occurring sequence. In some embodiments, the present invention provides engineered mosaic influenza HA polypeptides, wherein the amino acid sequence comprises SEQ ID NO: 40.

In one aspect, the present invention provides an engineered mosaic influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 41, wherein the amino acid sequence is not a naturally-occurring sequence.

In some embodiments, the present invention provides engineered mosaic influenza HA polypeptides, wherein the amino acid sequence comprises SEQ ID NO: 41.

In one aspect, the present invention provides an engineered mosaic influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 42, wherein the amino acid sequence is not a naturally-occurring sequence. In some embodiments, the present invention provides engineered mosaic influenza HA polypeptides, wherein the amino acid sequence comprises SEQ ID NO: 42.

In one aspect, the present invention provides an engineered mosaic influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 43, wherein the amino acid sequence is not a naturally-occurring sequence. In some embodiments, the present invention provides engineered mosaic influenza HA polypeptides, wherein the amino acid sequence comprises SEQ ID NO: 43.

In one aspect, the present invention provides an engineered mosaic influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 44 wherein the amino acid sequence is not a naturally-occurring sequence. In some embodiments, the present invention provides engineered mosaic influenza HA polypeptides, wherein the amino acid sequence comprises SEQ ID NO: 44.

In one aspect, the present invention provides an engineered mosaic influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 45, wherein the amino acid sequence is not a naturally-occurring sequence. In some embodiments, the present invention provides engineered mosaic influenza HA polypeptides, wherein the amino acid sequence comprises SEQ ID NO: 45.

In related aspects, the present invention provides an isolated nucleic acid molecule encoding an engineered mosaic HA polypeptide according to various methods described herein. In some embodiments, the nucleic acid molecule is codon-optimized for expression in mammalian cells, microalgae, plants, yeast, fungi or bacteria. In some embodiments, the present invention provides a vector comprising the nucleic acid sequence encoding an engineered mosaic HA polypeptide described herein. In some embodiments, the present invention provides an isolated cell comprising a vector encoding an engineered mosaic HA polypeptide described herein. In some embodiments, the cell is a mammalian cell.

In other aspects, the present invention provides a fusion protein comprising an engineered mosaic HA polypeptide described herein.

In further aspect, the present invention provides a vaccine composition comprising an engineered mosaic HA polypep-

tion or a fusion protein thereof described herein. In some embodiments, the vaccine composition is a split inactivated virus.

In still other aspects, the present invention provides a method of immunizing a subject against influenza virus, comprising administering to the subject a vaccine composition comprising an engineered mosaic HA polypeptide or a fusion protein thereof. In some embodiments, the pharmaceutical composition further comprises an adjuvant. In some embodiments, a pharmaceutical composition, an influenza HA polypeptide, a fusion protein or an influenza VLP or split inactivated virus thereof as described herein, is administered intramuscularly, intranasally, intradermally, subcutaneously, orally, or intravenously.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWING

The Drawing included herein, which is comprised of the following Figures, is for illustration purposes only not for limitation.

FIG. 1 shows a general illustration of the approach for designing engineered hemagglutinin (HA) polypeptides via structural mapping of antigenic repertoires. Mosaic antigen designs are constructed into a single HA molecule based on combinations of epitope patterns observed in HA sequences from circulating strains. Engineered HA molecules as described herein contain repertoires of neutralizing epitopes and antigenic sites or surface regions, which are computationally assembled from diverse strains. The epitopic regions are then selected which collectively elicit broadly neutralizing antibodies in a host.

FIG. 2 shows an exemplary flowchart for the design and production of engineered antigenic polypeptides.

FIG. 3 shows an exemplary flowchart for the design and production of engineered antigenic polypeptides using the collapse epitopes process.

FIG. 4 shows an exemplary flowchart for the design and production of engineered antigenic polypeptides using the dominant epitopes process.

FIG. 5 shows a plot of the first two principal components from a principal component analysis of the pairwise sequence identity matrix enabling the identification of clear patterns associated with influenza B hemagglutinins including the two influenza B lineages (Yamagata vs Victoria). Additionally, sequences form distinct clusters based on similarity. Reference and vaccine strains are highlighted as circles and arrows respectively, in FIG. 5 as are engineered SMART HA sequences (grey diamonds) which show little overlap with the clusters consisting of circulating strain HA sequences.

FIG. 6 shows an exemplary flowchart for the design and production of engineered HA polypeptides using structural mapping of antigenic repertoires. Antigenic repertoires of multiple epitopes were identified and organized into alignment coverage optimized repertoire subsets. Mosaic sequences were generated through combination of different epitopes. Mosaic combinations of epitopes were evaluated for alignment coverage based on geographic regions, viral isolate years, viral sub-family clusters or other measures to identify high scoring designs. Generated mosaic sequences were optimized by structural refinement and could be further refined through targeted sequence modifications.

FIG. 7 shows an exemplary flowchart for the design and production of engineered mosaic antigenic polypeptides.

FIG. 8 shows an exemplary overview of the four distinct SMART pattern selection processes.

FIG. 9 shows an exemplary workflow of the SMART Dominant workflow to design engineered mosaic antigenic polypeptides.

FIG. 10 demonstrates an exemplary workflow to input SMART design sequences into a fixed backbone model and subsequently score and select the engineered antigenic polypeptides.

FIG. 11 demonstrates the Rosetta energy function used to score and/or select engineered mosaic antigenic polypeptides. The native conformation is usually the lowest energy conformation.

FIG. 12 shows an exemplary scatter plot of total energy score vs model number. The lowest energy models are selected for further screening, analysis, and development.

FIG. 13 shows a schematic of the re-engineered transmembrane and cytoplasmic domains (SEQ ID NO: 63) which have been replaced to facilitate soluble expression in a mammalian host compatible with a Protein Expression and Purification Platform (PEPP) system.

FIG. 14 shows the expression of re-engineered influenza B HA designs. Approximately half of the recombinant HAs tested were expressed above threshold in a range from 41-320 µg/ml.

FIG. 15 shows verification of representative influenza B rHAs recognition by an anti-influenza B HA monoclonal antibody. Verification of binding to conformational epitopes was performed using Bio-Layer Interferometry (BLI) using a ForteBio Octet instrument. A low off-rate indicated by the shape of the curve suggests stronger binding of the rHA to the monoclonal antibody as opposed to a quick fall off which would indicate weaker binding.

FIG. 16 shows a detailed sequence overview of the mosaic approach. "MSA" sequences (from top to bottom): SEQ ID NO: 64-84; "MSA epitopes" (from top to bottom): SEQ ID NO: 85-105; "Unique epitope sets" (from top to bottom): SEQ ID NO: 106-115; "Epitope cassettes" (from left to right and top to bottom): SEQ ID NO: 116-130; "Other epitope cassettes" (in order of appearance): SEQ ID NO: 131-152.

FIG. 17A shows an exemplary comparison of overlapping H1N1 HA epitopes. "VDGW": SEQ ID NO: 153.

FIG. 17B shows an exemplary alignment of overlapping H1N1 HA epitopes. From top to bottom: SEQ ID NO: 154-163.

FIG. 18 shows an exemplary flowchart for the design and production of engineered mosaic antigenic polypeptides using the random epitopes process.

FIG. 19 shows an exemplary flowchart for the design and production of engineered mosaic antigenic polypeptides using the mosaic coverage process.

FIG. 20 shows an exemplary principal component analysis plot used to identify H1N1 HA clusters.

FIG. 21 shows representative serum titer induced against H1N1 influenza strains as measured in a hemagglutination inhibition (HAI) assay for selected engineered VLPs. Animals were immunized as described in Example 3 and serum collected on day 35 (14 days after boosting dose) was analyzed for its ability to inhibit hemagglutination mediated by various H1N1 influenza strains. Titer is defined as the maximum serum dilution resulting in complete inhibition of hemagglutination in 50% of the wells assayed. Each bar represents the virus-specific serum HAI titer induced by selected influenza VLPs or vehicle. The dotted line (DL) on

the graph represents a 1:40 HAI titer, which is known as the minimum HAI titer required for protection against a given influenza strain.

FIG. 22 shows representative serum titer induced against H1N1 influenza strains as measured in a microneutralization (MN) assay for selected engineered VLPs. Animals were immunized as described in Example 3 and serum collected on day 35 (14 days after the boosting dose) was analyzed for its ability to block infection with various H1N1 influenza strains. The MN titer is defined as the maximum serum dilution resulting in complete inhibition of infection with the indicated influenza strain in 50% of the wells tested. Each bar represents the virus-specific serum MN titer induced by selected influenza VLPs or vehicle. The dotted line on the graph represents a 1:80 MN titer, which is suggested is a minimum titer required for protection against influenza challenge.

FIG. 23 shows representative serum titer induced against influenza B strains as measured in a hemagglutination inhibition (HAI) assay for selected recombinant HA polypeptides. Animals were immunized as described in Example 4 and serum collected on day 56 (14 days after final boosting dose) was analyzed for its ability to inhibit hemagglutination mediated by various influenza B strains. Titer is defined as the maximum serum dilution resulting in complete inhibition of hemagglutination in 50% of the wells assayed. The strains selected for analysis included B/Florida/4/2006, B/Massachusetts/02/2012, B/Brisbane/60/2008, B/Wisconsin/1/2010, B/Texas/06/2011, B/Phuket/3073/2013. Each bar represents the virus-specific serum HAI titer induced by selected influenza recombinant HA polypeptide or vehicle. The first dotted line on the graph represents a 1:40 HAI titer, which is known as the minimum HAI titer required for protection against a given influenza strain. The second dotted line indicates 1:80 HAI titer which is double the suggested minimum titer required for protection against influenza challenge.

DEFINITIONS

In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth through the specification.

As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus for example, a reference to “a method” includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

Adjuvant: As used herein, the term “adjuvant” refers to a substance or vehicle that non-specifically enhances the immune response to an antigen. Adjuvants can include a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; or water-in-oil emulsion in which antigen solution is emulsified in mineral oil (for example, Freund’s incomplete adjuvant), sometimes with the inclusion of killed mycobacteria (Freund’s complete adjuvant) to further enhance antigenicity. Immunostimulatory oligonucleotides (such as those including a CpG motif) can also be used as adjuvants (for example, see U.S. Pat. Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; 6,339,068; 6,406,705; and 6,429,199). Adjuvants also include biological molecules, such as costimulatory molecules. Exemplary biological adjuvants include IL-2,

RANTES, GM-CSF, TNF- α , IFN- γ , G-CSF, LFA-3, CD72, B7-1, B7-2, OX-40L and 41 BBL.

Administer: As used herein, “administering” a composition to a subject means to give, apply or bring the composition into contact with the subject. Administration can be accomplished by any of a number of routes, such as, for example, topical, oral, subcutaneous, intramuscular, intraperitoneal, intravenous, intrathecal and intradermal.

Affinity: As is known in the art, “affinity” is a measure of the tightness with a particular ligand (e.g., an HA polypeptide) binds to its partner (e.g., an HA receptor, one or more glycans, etc.). Affinities can be measured in different ways. In some embodiments, affinity is measured by a quantitative assay (e.g., glycan binding assays). In some such embodiments, binding partner concentration (e.g., HA receptor, glycan, etc.) may be fixed to be in excess of ligand (e.g., an HA polypeptide) concentration so as to mimic physiological conditions (e.g., viral HA binding to cell surface glycans). In some embodiments, binding partner concentration may be fixed to so that ligand is in excess. Alternatively or additionally, in some embodiments, binding partner (e.g., HA receptor, glycan, etc.) concentration and/or ligand (e.g., an HA polypeptide) concentration may be varied. In some embodiments, affinity is assessed over a range of concentrations (e.g., serial dilutions) of ligand and/or of binding partner. In some embodiments, affinity (e.g., binding affinity) may be compared to a reference (e.g., a wild type HA that mediates infection of a humans) under comparable conditions (e.g., concentrations).

Animal: As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

Antibody: As used herein, the term “antibody” refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. In some embodiments, as used herein, the term “antibody” also refers to an “antibody fragment” or “antibody fragments”, which includes a portion of an intact antibody, such as, for example, the antigen-binding or variable region of an antibody. Examples of “antibody fragments” include Fab, Fab', F(ab')₂, and Fv fragments; triabodies; tetrabodies; linear antibodies; single-chain antibody molecules; and CDR-containing moieties included in multi-specific antibodies formed from antibody fragments. Those skilled in the art will appreciate that the term “antibody fragment” does not imply and is not restricted to any particular mode of generation. An antibody fragment may be produced through use of any appropriate methodology, including but not limited to cleavage of an intact antibody, chemical synthesis, recombinant production, etc. As is known in the art, intact antibodies as produced in nature are approximately 150 kDa tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kDa each) and two identical light chain polypeptides (about 25 kDa each) that associate with each other into what is commonly referred to as a “Y-shaped” structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)—an amino-terminal variable

11

(V_H) domain (located at the tips of the Y structure), followed by three constant domains: C_H1, C_H2, and the carboxy-terminal C_H3 (located at the base of the Y's stem). A short region, known as the "switch", connects the heavy chain variable and constant regions. The "hinge" connects C_H2 and C_H3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains—an amino-terminal variable (V_L) domain, followed by a carboxy-terminal constant (C_L) domain, separated from one another by another "switch". Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the C_H2 domain. Each domain in a natural antibody has a structure characterized by an "immunoglobulin fold" formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as "complement determining regions" (CDR1, CDR2, and CDR3) and four somewhat invariant "framework" regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. Amino acid sequence comparisons among antibody polypeptide chains have defined two light chain (κ and λ) classes, several heavy chain (e.g., μ, γ, α, ε, δ) classes, and certain heavy chain subclasses (α1, α2, γ1, γ2, γ3, and γ4). Antibody classes (IgA [including IgA1, IgA2], IgD, IgE, IgG [including IgG1, IgG2, IgG3, IgG4], IgM) are defined based on the class of the utilized heavy chain sequences. For purposes of the present invention, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an "antibody", whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is monoclonal; in some embodiments, an antibody is polyclonal. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, an antibody sequence elements are humanized, primatized, chimeric, etc., as is known in the art. Moreover, the term "antibody" as used herein, will be understood to encompass (unless otherwise stated or clear from context) can refer in appropriate embodiments to any of the art-known or developed constructs or formats for capturing antibody structural and functional features in alternative presentation. For example, in some embodiments, the term can refer to bi- or other multi-specific (e.g., zybodies, etc.) antibodies, Small Modular ImmunoPharmaceuticals ("SMIPsTM"), single chain antibodies, camelid antibodies, and/or antibody fragments. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a gly-

12

can, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc.], or other pendant group [e.g., poly-ethylene glycol, etc.]).

Antigen: As used herein, the term "antigen", refers to an agent that elicits an immune response; and/or (ii) an agent that is bound by a T cell receptor (e.g., when presented by an MHC molecule) or to an antibody (e.g., produced by a B cell) when exposed or administered to an organism. In some embodiments, an antigen elicits a humoral response (e.g., including production of antigen-specific antibodies) in an organism; alternatively or additionally, in some embodiments, an antigen elicits a cellular response (e.g., involving T-cells whose receptors specifically interact with the antigen) in an organism. It will be appreciated by those skilled in the art that a particular antigen may elicit an immune response in one or several members of a target organism (e.g., mice, rabbits, primates, humans), but not in all members of the target organism species. In some embodiments, an antigen elicits an immune response in at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% of the members of a target organism species. In some embodiments, an antigen binds to an antibody and/or T cell receptor, and may or may not induce a particular physiological response in an organism. In some embodiments, for example, an antigen may bind to an antibody and/or to a T cell receptor in vitro, whether or not such an interaction occurs in vivo. In some embodiments, an antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. In some embodiments of the disclosed compositions and methods, influenza HA protein is an antigen.

Approximately: As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

Associated with: Two events or entities are "associated" with one another, as that term is used herein, if the presence, level and/or form of one is correlated with that of the other. For example, a particular entity (e.g., polypeptide) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of and/or susceptibility of the disease, disorder, or condition (e.g., across a relevant population). In some embodiments, two or more entities are physically "associated" with one another if they interact, directly or indirectly, so that they are and remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

Binding: It will be understood that the term "binding", as used herein, typically refers to a non-covalent association between or among two or more entities. "Direct" binding involves physical contact between entities or moieties; indirect binding involves physical interaction by way of physical

contact with one or more intermediate entities. Binding between two or more entities can be assessed in any of a variety of contexts—including where interacting entities or moieties are studied in isolation or in the context of more complex systems (e.g., while covalently or otherwise associated with a carrier entity and/or in a biological system or cell).

Carrier: As used herein, the term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which a composition is administered. In some exemplary embodiments, carriers can include sterile liquids, such as, for example, water and oils, including oils of petroleum, animal, vegetable or synthetic origin, such as, for example, peanut oil, soybean oil, mineral oil, sesame oil and the like. In some embodiments, carriers are or include one or more solid components.

Characteristic Portion: As used herein, the term “characteristic portion” is used, in the broadest sense, to refer to a portion of a substance whose presence (or absence) correlates with presence (or absence) of a particular feature, attribute, or activity of the substance. In some embodiments, a characteristic portion of a substance is a portion that is found in the substance and in related substances that share the particular feature, attribute or activity, but not in those that do not share the particular feature, attribute or activity.

Characteristic sequence element: As used herein, the phrase “characteristic sequence element” refers to a sequence element found in a polymer (e.g., in a polypeptide or nucleic acid) that represents a characteristic portion of that polymer. In some embodiments, presence of a characteristic sequence element correlates with presence or level of a particular activity or property of the polymer. In some embodiments, presence (or absence) of a characteristic sequence element defines a particular polymer as a member (or not a member) of a particular family or group of such polymers. A characteristic sequence element typically comprises at least two monomers (e.g., amino acids or nucleotides). In some embodiments, a characteristic sequence element includes at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, or more monomers (e.g., contiguously linked monomers). In some embodiments, a characteristic sequence element includes at least first and second stretches of continuous monomers spaced apart by one or more spacer regions whose length may or may not vary across polymers that share the sequence element.

Codon-optimized: As used herein, a “codon-optimized” nucleic acid sequence refers to a nucleic acid sequence that has been altered such that translation of the nucleic acid sequence and expression of the resulting protein is improved optimized for a particular expression system. A “codon-optimized” nucleic acid sequence encodes the same protein as a non-optimized parental sequence upon which the “codon-optimized” nucleic acid sequence is based. For example, a nucleic acid sequence may be “codon-optimized” for expression in mammalian cells (e.g., CHO cells, human cells, mouse cells etc.), bacterial cells (e.g., *E. coli*), insect cells, yeast cells or plant cells.

Comparable: The term “comparable”, as used herein, refers to two or more agents, entities, situations, sets of conditions, etc. that may not be identical to one another but that are sufficiently similar to permit comparison there between so that conclusions may reasonably be drawn based on differences or similarities observed. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc. to be considered comparable.

Corresponding to: As used herein, the term “corresponding to” is often used to designate the position/identity of an amino acid residue in a polypeptide of interest (e.g., an HA polypeptide). Those of ordinary skill will appreciate that, for purposes of simplicity, residues in a polypeptide are often designated using a canonical numbering system based on a reference related polypeptide, so that an amino acid “corresponding to” a residue at position 190, for example, need not actually be the 190th amino acid in a particular amino acid chain but rather corresponds to the residue found at 190 in the reference polypeptide; those of ordinary skill in the art readily appreciate how to identify “corresponding” amino acids. Typically, residues in HA polypeptides are designated with reference to a canonical wild type HA, and reference in a polypeptide of interest that correspond to residues in the canonical wild type HA are described using the numbering of the residues to which they correspond.

Determine: Many methodologies described herein include a step of “determining” Those of ordinary skill in the art, reading the present specification, will appreciate that such “determining” can utilize any of a variety of techniques available to those skilled in the art, including for example specific techniques explicitly referred to herein. In some embodiments, a determination involves manipulation of a physical sample. In some embodiments, a determination involves consideration and/or manipulation of data or information, for example utilizing a computer or other processing unit adapted to perform a relevant analysis. In some embodiments, a determination involves receiving relevant information and/or materials from a source. In some embodiments, determining involves comparing one or more features of a sample or entity to a comparable reference.

Engineered: The term “engineered”, as used herein, describes a polypeptide whose amino acid sequence has been designed by man and/or whose existence and production require human intervention and/or activity. For example, an engineered HA polypeptide has an amino acid sequence that is intentionally designed to elicit a particular effect and that differs from the amino acid sequences of HA polypeptides found in natural influenza isolates. In some embodiments, an engineered HA polypeptide has an amino acid sequence that differs from the amino acid sequence of HA polypeptides included in the NCBI database.

Epitope: As used herein, the term “epitope” includes any moiety that is specifically recognized by an immunoglobulin (e.g., antibody or receptor) binding component in whole or in part. In some embodiments, an epitope is comprised of a plurality of amino acid residues in an antigen. In some embodiments, the amino acid residues are surface-exposed when the antigen adopts a relevant three-dimensional conformation. In some embodiments, the amino acid residues are physically near to or continuous with each other in space when the antigen adopts such a conformation. In some embodiments, at least some of the amino acids are physically separated from one another when the antigen adopts an alternative conformation (e.g., is linearized; e.g., a non-linear epitope).

Excipient: As used herein, the term “excipient” refers to a non-therapeutic agent that may be included in a pharmaceutical composition, for example to provide or contribute to a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

Expression: The term “expression” or “expressed”, when used in reference to a nucleic acid herein, refers to one or more of the following events: (1) production of an RNA transcript of a DNA template (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end formation); (3) translation of an RNA into a polypeptide; and/or (4) post-translational modification of a polypeptide.

Fusion protein: As used herein, the term “fusion protein” refers to a protein encoded by a nucleic acid sequence engineered from nucleic acid sequences encoding at least a portion of two different (e.g., heterologous) proteins. As persons of skill are no doubt aware, to create a fusion protein nucleic acid sequences are joined such that the resulting reading does not contain an internal stop codon. In some embodiments, fusion proteins as described herein include an influenza HA polypeptide or fragment thereof.

Hemagglutinin (HA) polypeptide: As used herein, the term “hemagglutinin polypeptide” (or “HA polypeptide”) refers to a polypeptide whose amino acid sequence includes at least one characteristic sequence of an influenza type A or B HA. A wide variety of HA sequences from influenza isolates are known in the art; indeed, the National Center for Biotechnology Information (NCBI) maintains a database (<http://www.ncbi.nlm.nih.gov/genomes/FLU/>) that, as of the filing of the present application includes approximately 40,000 HA sequences (for type A and B viruses). Those of ordinary skill in the art, referring to this database, can readily identify sequences that are characteristic of HA polypeptides generally, and/or of particular HA polypeptides (e.g., H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, or H16 polypeptides; or of HAs that mediate infection of particular hosts, e.g., human, avian, seal etc.). For example, in some embodiments, an HA polypeptide includes one or more characteristic sequence elements found between about residues 97 and about 185, about 324 and about 340, about 96 and about 100, and/or about 130 and about 230 of an HA protein found in a natural isolate of an influenza virus.

Host: The term “host” is used herein to refer to a system (e.g., a cell, organism, etc.) in which a polypeptide of interest is present. In some embodiments, a host is a system that is susceptible to infection with a particular infectious agent. In some embodiments, a host is a system that expresses a particular polypeptide of interest.

Host cell: As used herein, the phrase “host cell” refers to a cell into which exogenous DNA (recombinant or otherwise) has been introduced. For example, host cells may be used to produce the engineered influenza hemagglutinin polypeptides described herein by standard recombinant techniques. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but, to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein. In some embodiments, host cells include any prokaryotic and eukaryotic cells suitable for expressing an exogenous DNA (e.g., a recombinant nucleic acid sequence). Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of *E. coli*, *Bacillus* spp., *Streptomyces* spp., etc.), mycobacteria cells, fungal cells, yeast cells (e.g., *S. cerevisiae*, *S. pombe*, *P. pastoris*, *P. methanolica*, etc.), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, *Trichoplusia ni*, etc.), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or

quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (e.g., CHO K1, DXB-11 CHO, Veggie-CHO), COS (e.g., COS-7), retinal cell, Vero, CV1, kidney (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes, e.g., a retinal cell that expresses a viral gene (e.g., a PER.C6™ cell).

Immune response: As used herein, the term “immune response” refers to a response of a cell of the immune system, such as a B cell, T cell, dendritic cell, macrophage or polymorphonucleocyte, to a stimulus such as an antigen or vaccine. An immune response can include any cell of the body involved in a host defense response, including for example, an epithelial cell that secretes an interferon or a cytokine. An immune response includes, but is not limited to, an innate and/or adaptive immune response. As used herein, a protective immune response refers to an immune response that protects a subject from infection (prevents infection or prevents the development of disease associated with infection). Methods of measuring immune responses are well known in the art and include, for example, measuring proliferation and/or activity of lymphocytes (such as B or T cells), secretion of cytokines or chemokines, inflammation, antibody production and the like.

Immunogen: As used herein, the term “immunogen” refers to a compound, composition, or substance which is capable, under appropriate conditions, of stimulating an immune response, such as the production of antibodies or a T cell response in an animal, including compositions that are injected or absorbed into an animal. As used herein, an “immunogenic composition” is an administrable composition comprising an immunogen (such as an HA polypeptide) “Immunogenic compositions” include, for example, vaccines. As used herein, “immunize” means to render a subject protected from an infectious disease, such as by vaccination.

In vitro: As used herein, the term “in vitro” refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, etc., rather than within a multi-cellular organism.

In vivo: As used herein, the term “in vivo” refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, in vitro systems).

Influenza virus: As used herein, the term “influenza virus” refers to a segmented negative-strand RNA virus that belongs to the Orthomyxoviridae family. There are three types of Influenza viruses, A, B, and C. Influenza B viruses have been reported to infect humans, seals, horses and swine. Human influenza A and B viruses (A and B viruses) are equally prevalent among human beings, yet most A virus genes evolve two to three times faster than the corresponding genes in B viruses. Most influenza B viruses cause mild localized infections of the respiratory and intestinal tract in humans. Influenza B causes the same spectrum of disease as influenza A but does not cause pandemics. In 2016, the WHO GISRS laboratories reported that of more than 80,000 samples tested, 35.7% were typed as influenza A and 64.3%

as influenza B. Of the characterized B viruses, 20.6% belonged to the B-Yamagata lineage and 79.4% to the B-Victoria lineage.

Influenza vaccine: As used herein, the term “influenza vaccine” refers to an immunogenic composition capable of stimulating an immune response, administered for the prevention, amelioration, or treatment of influenza virus infection. An influenza vaccine may include, for example, attenuated or killed influenza virus, virus-like particles (VLPs) and/or antigenic polypeptides (e.g., the engineered hemagglutinins described herein) or DNA derived from them, or any recombinant versions of such immunogenic materials.

Isolated: As used herein, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured with human intervention. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered “isolated” or even “pure”, after having been combined with certain other components such as, for example, one or more carriers or excipients (e.g., buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polynucleotide that occurs in nature is considered to be “isolated” when, a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some embodiments, a polypeptide that is chemically synthesized or is synthesized in a cellular system different from that which produces it in nature is considered to be an “isolated” polypeptide. Alternatively or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered to be an “isolated” polypeptide to the extent that it has been separated from other components a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

Nucleic acid: As used herein, the phrase “nucleic acid”, in its broadest sense, refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some embodiments, “nucleic acid” refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides); in some embodiments, “nucleic acid” refers to an oligonucleotide chain comprising individual nucleic acid

residues. In some embodiments, a “nucleic acid” is or comprises RNA; in some embodiments, a “nucleic acid” is or comprises DNA. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, a nucleic acid analog differs from a nucleic acid in that it does not utilize a phosphodiester backbone. For example, in some embodiments, a nucleic acid is, comprises, or consists of one or more “peptide nucleic acids”, which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. Alternatively or additionally, in some embodiments, a nucleic acid has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine). In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a nucleic acid comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, a nucleic acid includes one or more introns. In some embodiments, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (in vivo or in vitro), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some embodiments, a nucleic acid is single stranded; in some embodiments, a nucleic acid is double stranded. In some embodiments a nucleic acid has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. In some embodiments, a nucleic acid has enzymatic activity.

Pandemic strain: A “pandemic” influenza strain is one that has caused or has capacity to cause pandemic infection of human populations. In some embodiments, a pandemic strain has caused pandemic infection. In some embodiments, such pandemic infection involves epidemic infection across multiple territories; in some embodiments, pandemic infection involves infection across territories that are separated from one another (e.g., by mountains, bodies of water, as part of distinct continents, etc.) such that infections ordinarily do not pass between them.

Pharmaceutically acceptable vehicles: The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. Remington's Pharmaceutical Sciences, by

E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compositions, such as one or more influenza vaccines, and additional pharmaceutical agents. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Prevention: The term “prevention”, as used herein, refers to prophylaxis, avoidance of disease manifestation, a delay of onset, and/or reduction in frequency and/or severity of one or more symptoms of a particular disease, disorder or condition (e.g., infection for example with influenza virus). In some embodiments, prevention is assessed on a population basis such that an agent is considered to “prevent” a particular disease, disorder or condition if a statistically significant decrease in the development, frequency, and/or intensity of one or more symptoms of the disease, disorder or condition is observed in a population susceptible to the disease, disorder, or condition.

Receptor-Binding Site (RBS): As used herein, the term “receptor-binding site” or “RBS” comprises contiguous or non-contiguous amino acid residues of the head region of an influenza HA polypeptide, which include amino acids involved in direct binding of sialic acid on the target cell receptor proteins. Amino acid residues that make up a “receptor-binding site” or “RBS” of an influenza HA polypeptide may be described from crystal structures of HA polypeptides complexed with sialic acid analogs and identifying amino acid residues within a certain proximity to the analog or may be described in reference to an HA polypeptide sequence from a particular viral strain (e.g., B/Victoria/02/1987, B/Yamagata/16/1988). Thus, in some embodiments, the “receptor-binding site” or “RBS” of an engineered HA polypeptide as described herein may be determined using a reference HA polypeptide sequence. In some embodiments, the “receptor-binding site” or “RBS” of an engineered HA polypeptide as described herein may be determined using the crystal structures of HA polypeptide sequence. An exemplary reference crystal structure of HA polypeptide sequence includes B/Yamanashi/166/1998 influenza B pdb14M40.

Recombinant: As used herein, the term “recombinant” is intended to refer to polypeptides (e.g., HA polypeptides as described herein) that are designed, engineered, prepared, expressed, created or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell, polypeptides isolated from a recombinant, combinatorial polypeptide library or polypeptides prepared, expressed, created or isolated by any other means that involves splicing selected sequence elements to one another. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements and/or combinations thereof is designed in silico. In

some embodiments, one or more such selected sequence elements results from the combination of multiple (e.g., two or more) known sequence elements that are not naturally present in the same polypeptide (e.g., two epitopes from two separate HA polypeptides).

Recombinant influenza vaccine: As used herein, the term “recombinant influenza vaccine” refers to influenza-specific immunogenic composition comprising one or more of the engineered influenza hemagglutinins described herein, including, but not limited to whole influenza virus, subunit preparations thereof, virus-like particles, recombinant protein (i.e., preparations composed of recombinant HA purified to varying degree), and DNA- and viral vector-based vaccines. Recombinant influenza vaccines as described herein may optionally contain one or more adjuvants.

Reference: The term “reference” is often used herein to describe a standard or control agent, individual, population, sample, sequence or value against which an agent, individual, population, sample, sequence or value of interest is compared. In some embodiments, a reference agent, individual, population, sample, sequence or value is tested and/or determined substantially simultaneously with the testing or determination of the agent, individual, population, sample, sequence or value of interest. In some embodiments, a reference agent, individual, population, sample, sequence or value is a historical reference, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference agent, individual, population, sample, sequence or value is determined or characterized under conditions comparable to those utilized to determine or characterize the agent, individual, population, sample, sequence or value of interest.

Specificity: As is known in the art, “specificity” is a measure of the ability of a particular ligand (e.g., an antibody, an HA polypeptide, etc.) to distinguish its binding partner (e.g., an antigen, a human HA receptor, and particularly a human upper respiratory tract HA receptor) from other potential binding partners (e.g., a seal HA receptor).

Subject: As used herein, the term “subject” means any mammal, including humans. In certain embodiments of the present invention the subject is an adult, an adolescent or an infant. In some embodiments, terms “individual” or “patient” are used and are intended to be interchangeable with “subject”. Also contemplated by the present invention are the administration of the pharmaceutical compositions and/or performance of the methods of treatment in-utero.

Substantially: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

Substantially Similar: As used herein, the term “substantially similar” refers to a comparison between two entities. In general, entities are considered to be “substantially similar” to one another when they share sufficient structural similarity (e.g., a characteristic structural feature) that they have a comparable likelihood of sharing one or more additional attributes or features. To give but one example, a characteristic, for example, glycosylation site pattern, being either the same or similar enough between two influenza strains, that the human pandemic risk of each strain is the same.

Substantial identity: The phrase “substantial identity” is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be “substantially identical” if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, *J. Mol. Biol.*, 215(3): 403-410, 1990; Altschul, et al., *Methods in Enzymology*; Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis et al., *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132)*, Humana Press, 1999. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues. In the context of an HA polypeptide, reference to “substantial identity” typically refers to a HA polypeptide (or HA epitope) having an amino acid sequence at least 90%, preferably at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of a reference HA polypeptide (or HA epitope).

Transformation: As used herein, refers to any process by which exogenous DNA is introduced into a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. In some embodiments, a particular transformation methodology is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, mating, lipofection. In some embodiments, a “transformed” cell is stably transformed in that the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. In some embodiments, a transformed cell transiently expresses introduced nucleic acid for limited periods of time.

Vaccination: As used herein, the term “vaccination” refers to the administration of a composition intended to generate an immune response, for example to a disease-causing agent. Vaccination can be administered before, during, and/or after exposure to a disease-causing agent, and/or to the development of one or more symptoms, and in some embodiments, before, during, and/or shortly after exposure to the agent. In some embodiments, vaccination includes multiple administrations, appropriately spaced in time, of a vaccinating composition.

Vector: As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector

is a “plasmid”, which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

Virus-like particle (VLP): As used herein, the phrase “virus-like particle” or “VLP” refers to particles that resemble a virus yet lack any viral genetic material and, therefore, are not infectious. A “virus-like particle” or “VLP” may be produced by heterologous expression in a variety of cell culture systems including mammalian cell lines, insect cell lines, yeast, and plant cells. In addition, VLPs can be purified by methods known in the art. In some embodiments, an influenza VLP as described herein comprises hemagglutinin (HA) polypeptides and neuraminidase (NA) polypeptides. In some embodiments, influenza VLPs as described herein comprise HA polypeptides, NA polypeptides and/or viral structural polypeptides (e.g., an influenza structural protein such as influenza M1). In some certain embodiments, influenza VLPs as described herein comprise HA polypeptides, NA polypeptides and/or M1 polypeptides. In some embodiments, influenza VLPs as described herein comprise HA polypeptides, NA polypeptides and/or HIVgag polypeptides. Influenza VLPs can be produced by transfection of host cells (e.g., mammalian cells) with plasmids encoding HA and NA proteins, and optionally HIVgag proteins. After incubation of the transfected cells for an appropriate time to allow for protein expression (such as for approximately 72 hours), VLPs can be isolated from cell culture supernatants. In some embodiments, influenza VLPs as described herein are produced by transient transfection in mammalian cells (e.g., human cells). In some embodiments, influenza VLPs are analyzed by the use of one or more assays. To give but a few examples, influenza VLPs may be analyzed for hemagglutinin activity, dynamic light scattering and hemmagglutinin content quantitation by protein staining. Other assays will be readily apparent to persons of skill upon reviewing the present disclosure.

Wild type: As is understood in the art, the phrase “wild type” generally refers to a normal form of a protein or nucleic acid, as is found in nature. For example, wild type HA polypeptides are found in natural isolates of influenza virus. A variety of different wild type HA sequences can be found in the NCBI influenza virus sequence database, available through the world wide web at ncbi.nlm.nih.gov/genomes/FLU/FLU.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

The present invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting unless indicated, since the scope of the present invention will be limited only by the appended claims.

Unless stated otherwise, all technical and scientific terms and phrases used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

Engineering Mosaic Influenza Antigens

Influenza viruses are members of the family Orthomyxoviridae and are divided into three genera, A, B, and C. Influenza A and B viruses cause respiratory infections in humans. Current vaccines are designed to induce immunity to hemagglutinin, one of two glycoproteins present on the surface of influenza viruses. Despite the availability of highly effective vaccines, influenza infection still results in up to 5,000,000 hospitalizations and 500,000 deaths annually worldwide. Currently available vaccines against influenza include up to four influenza hemagglutinin components intended to provide protection against H1N1, H3N2, and influenza B strains. Vaccine compositions are reassessed annually by the World Health Organization (WHO) to accommodate antigenic shift and drift in circulating virus strains. Such a strategy requires diligent surveillance of circulating influenza strains from year to year, and vaccine mismatches resulting from inaccurate predictions or unpredictable HA mutations arising during vaccine manufacture, which can result in increased morbidity and mortality even in vaccinated populations. Given the shortcomings of the currently available vaccines, a key goal of influenza research is to develop vaccination approaches that provide greater efficacy against mismatched strains.

Structural Mapping of Antigenic Repertoires

One rational design approach to creating a broadly protective influenza vaccine is to engineer antigens that include epitopes from as many viral isolates as possible. According to the present invention, generation of mosaic epitope sequences, in particular—B-cell epitope sequences—can be achieved using a methodology termed SMART for the 'Structural Mapping of Antigenic Repertoires'. The present invention is based, in part, on the recognition that a rational design approach to creating a broadly protective influenza vaccine can be developed by providing engineered influenza antigenic polypeptides (for example, hemagglutinin, neuraminidase, M2e, etc.) that include epitopes from multiple viral isolates in a polyvalent vaccine (FIG. 1). The designs, in some embodiments, are based on combinations of multiple B cell epitopes and antigenic regions from different hemagglutinin sequences into mosaic antigens. These mosaic epitope antigens, in some embodiments, are predicted to confer cross-protection against multiple influenza B lineage strains by maximizing sequence homology for at least one neutralizing epitope.

In some embodiments, a method of engineering a mosaic influenza hemagglutinin (HA) polypeptide according to the SMART methodology comprises the steps of: (1) obtaining HA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus; (2) aligning the HA amino acid sequences to generate an alignment; identifying the positions of amino acids comprising known epitopes and antigenic regions; (3) compiling the amino acid residues across the alignment at the identified positions for each epitope and antigenic region; (3) defining a set of amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino sequence pattern in the set is represented only once; (4) selecting a sequence from the set for each epitope or antigenic region; and (5) inserting one or more selected sequences into corresponding locations in a structural backbone of HA to generate a mosaic influenza HA polypeptide. Alternatively, instead of selecting a unique sequence at step (4), a consensus sequence is defined from each set for each epitope or antigenic region.

Hemagglutinin (HA) is responsible for the binding of influenza virus to target cells with sialic acid on their membranes. Over time, HA accumulates mutations in its sequence in a process termed 'antigenic drift', allowing the virus to evade the human immune response. Although Influenza B viruses mutate at a lower rate than Influenza A viruses, the rate of mutation is fast enough that periodic reformulation of the Influenza B component of vaccines is required. Additionally, Influenza B virus has diverged into two antigenically distinct lineages (Yamagata and Victoria).

Current vaccines are designed to protect against the predominant circulating strains of Influenza A (subtype H1N1 and H3N2) and Influenza B. Previously, the Influenza B component of influenza vaccines was specific for a single lineage; with the introduction of quadrivalent influenza vaccine (QIV), both Influenza B lineages are now represented in a single vaccine. Accumulating mutations in HA reduce the effectiveness of any existing vaccine to protect against future circulating influenza viruses. Consequently, currently marketed influenza vaccines must be reviewed annually and updated if necessary. The development of a broadly protective vaccine antigen that tolerates some amount of antigenic drift holds the promise to increase the breadth of response as compared to current vaccines, which are more strain-specific. Additionally, a broadly protective Influenza B HA should provide a cross protective immune response against both Influenza B lineages, Yamagata and Victoria.

In some embodiments, engineered HA polypeptides as described herein achieve a greater efficacy against mismatched strains and/or strains associated with an increased morbidity and/or mortality. In some embodiments, engineered HA polypeptides as described herein provide enhanced protection against influenza during mismatch years thereby reducing reliance on accurate strain predictions from year to year. In some embodiments, engineered HA polypeptides as described herein are used in vaccines and allow for less frequent immunizations by providing sufficient breadth of immunity to cover antigenic drift that generally accumulates between seasons of influenza infection.

Engineered hemagglutinin (HA) polypeptides are molecular entities that specifically elicit an immune response in a subject. Such engineered HA polypeptides find a variety of uses in the art, including prophylactic and therapeutic uses. Engineered HA polypeptides of the present invention, in some embodiments, address the lack of breadth

and cross-protection observed in current influenza vaccine formulations. For example, in some embodiments, engineered HA polypeptides as described herein provide a cross protective immune response against both Influenza B lineages, Yamagata and Victoria. In some embodiments, engineered HA polypeptides as described herein may be used alone or in combination with other influenza antigens. In some embodiments, engineered HA polypeptides of the present invention may be used as a component of seasonal influenza vaccines or as part of influenza vaccination regimens intended to confer long-lasting, multi-season protection.

Various techniques may be used to obtain, align and select influenza sequences for each epitope or antigenic region. For example, Principal Components Analysis (PCA) is a common technique for working with high dimensional data and highlighting patterns in the data (i.e. it can be used to simplify large datasets and facilitate data exploration and visualization). Applied to biological sequences (proteins, genes), the technique enables comparison of thousands of sequences and the identification of groups of similar sequences based on a measure of sequence dissimilarity (Hamming distance, percent identity, percent similarity, surface accessibility, etc.). In the case of Human influenza viruses, influenza antigen protein sequences may be obtained from all publicly available amino acid sequences including, but not limited to, those available HA or NA amino acid sequences in the NCBI Influenza Virus Resource database. In some embodiments, more than 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 12,000, 14,000, or 15,000 HA or NA amino acid sequences from multiple circulating influenza strains are obtained. In some embodiments, 100-5000, 100-6000, 100-7000, 100-8000, 100-9000, 100-10,000, 100-15,000, or 100-20,000 HA or NA amino acid sequences from multiple circulating influenza strains are obtained.

In some embodiments, HA or NA protein sequences are obtained from more than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or 100 circulating influenza strains. In some embodiments, HA or NA amino acid sequences utilized in the present invention are obtained of a particular type and/or subtype of influenza virus. For example, HA or NA amino acid sequences may be obtained from a type A influenza virus. In some embodiments, the HA protein of an influenza A virus is selected from subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, or H16. In some embodiments, the influenza virus is selected from the group consisting of H1N1, H3N2, H5N1, and H7N9. In some embodiments, the type A virus is a seasonal strain, such as, /Texas/36/1991, A/Singapore/1986, A/New Caledonia/20/1999, A/Solomon Islands/03/2006, A/Brisbane/59/2007, or A/Wisconsin/67/2005. In some embodiments, the type A virus is a pandemic strain such as A/California/07/2009, A/California/04/2009, A/Belgium/145/2009, A/South Carolina/01/1918, or A/New Jersey/1976.

In some embodiments, HA or NA amino acid sequences may be obtained from a type B influenza virus. For example, the influenza B virus is a Yamagata lineage strain. In some embodiments, the influenza B virus is a Victoria lineage strain. In some embodiments, HA or NA amino acid sequences are obtained from an influenza B virus such as B/Hong Kong/330/2001, B/Hong Kong/05/1972, B/Lee/40, B/Massachusetts/02/2012, B/Panama/45/1990, B/Singapore/222/79, B/Victoria/02/1987, B/Yamagata/16/1988, or B/Brisbane/60/2008.

Typically, HA or NA amino acid sequences are trimmed to remove signal peptides, transmembrane regions and cyto-

plasmic tails, and the resulting ectodomain sequences are aligned. In some embodiments, redundant sequences are removed before alignment by screening the HA or NA amino acid sequences for those with 100%, or greater than 99.9%, 99%, 98%, 97%, or 96% sequence identity, sequence identity, and removing all but one of the redundant sequences.

Visualization of principal components may be used to identify patterns associated with influenza B hemagglutinins including the two influenza B lineages (Yamagata vs Victoria). Additionally, sequences form distinct clusters based on similarity.

Modifications designed into the engineered HAs or NAs is deduced from an in silico analysis of sequence variation in both past and current circulating influenza strains. This analysis includes mapping antigenic and epitope patterns as well as structural modeling of the HA or NA protein. Targeted changes are subsequently introduced at corresponding amino acid residue locations and/or specific regions of the protein with known immune profiles in order to yield novel influenza B HA or NA polypeptides that would be reactive across the sequence clusters.

Each novel mosaic design is composed of multiple neutralizing HA or NA B-cell epitope patterns derived from antigenically diverse influenza B strains (including both Yamagata and Victoria lineages). The mosaic pattern of B-cell epitopes is assembled onto a backbone hemagglutinin or neuraminidase sequence. As non-limiting examples, suitable backbone hemagglutinin or neuraminidase sequences may be derived from: B/Hong Kong/330/2001, B/Hong Kong/05/1972, B/Lee/40, B/Massachusetts/02/2012, B/Panama/45/1990, B/Singapore/222/79, B/Victoria/02/1987, B/Yamagata/16/1988, B/Brisbane/60/2008. However, other backbone sequences could also be contemplated for this invention. The selected backbone provides the inter-epitope sequence of the engineered construct as well as the signal peptide and transmembrane domains required for full-length hemagglutinin molecules that are expressed and functional.

Exemplary SMART Workflows

Briefly, known neutralizing and non-neutralizing hemagglutinin B-cell epitopes described for any influenza A and B virus are mapped to corresponding regions of Influenza B HA or NA. Individual sequences for each mapped B-cell epitope site are extracted and enumerated from the sequences of all available circulating Influenza B strains to generate an 'antigenic repertoire'. Individual sequences corresponding to different antigenic regions of HA or NA can be selected (or, alternatively, distilled into a consensus sequence) from the antigenic repertoire for each mapped epitope site and combined into novel mosaic pattern antigens (for example, epitope site 1 from circulating strain X, epitope site 2 from circulating strain Y, epitope site 3 from circulating strain Z, etc.) on a particular backbone strain, wherein the combination may be chosen or selected to elicit a particular immune response. Four distinct SMART workflows are developed to combine antigenic repertoires into novel mosaic hemagglutinin molecules that are distinct from natural circulating strains. An overview of the compiled SMART workflow is presented in FIG. 2.

Four non-limiting exemplary SMART workflows are:

1. SMART Random: B-cell epitope sequences for each of the mapped epitope sites are combined at random (drawn from all unique sequences for each epitope site in the antigenic repertoire). The resulting random combinations are scored for coverage of naturally occurring strains (by year, geography, and sequence coverage) and the highest

scoring combinations are selected. The highest scoring selections based on breadth of coverage across naturally occurring strains by year, geography and sequence cluster are then combined with one a backbone sequence

2. SMART Choice: Unique epitope sequences for each mapped epitope site are combined using an algorithm to maximize breadth of coverage across naturally occurring strains. An initial seed epitope is selected, and for each additional epitope added to the mosaic, a sequence is selected from the antigenic repertoire of the site that maximizes the breadth of coverage (i.e. as many naturally occurring strains as possible matched by at least one epitope). The highest scoring selections based on breadth of coverage across naturally occurring strains by year, geography and sequence cluster are then combined into one backbone sequence

3. SMART Collapsed: A consensus sequence for each epitope is defined from the unique antigenic repertoire for that epitope site. The consensus sequences for each distinct epitope site are combined into a single mosaic pattern for the antigen and merged into a backbone sequence. This approach determines the consensus of unique epitope sequences for all epitope sites, combines the epitope consensus sequence into a mosaic pattern template, and combines the mosaic pattern template with desired backbone sequence(s).

The collapsed epitopes approach generates a single sequence for each mapped epitope site in the mosaic using a consensus approach. The unique amino acid residues comprising epitope sequences are identified from the antigenic repertoire. The most common amino acid at a given position for each mapped epitope site is used in the mosaic template pattern. The data flow and method steps are overviewed in FIG. 3. An upper case letter is used for residues that occur in 75+% of the unique epitope patterns; otherwise a lower case letter is used. The “collapse_epitopes” step takes as input the epitopes and columns files. This step also integrates an ordered list of epitope names that specify the processing order for epitopes to be added to the mosaic template sequence. The program generates a FASTA formatted mosaic template sequence named “_collapsed_”. The merge_seqs step replaces gap characters in the mosaic template sequence with the corresponding residue from the backbone sequence.

4. SMART Dominant: For each epitope the unique sequences are ordered by their dominance as determined by the number of naturally occurring strains represented by that sequence. Dominant sequences for each epitope are selected and grafted onto a backbone in decreasing order of dominance (i.e. most dominant epitope patterns, then the second most dominant patterns, and so on). The SMART Dominant approach generates polyvalent B-cell vaccine proteins using the occurrence frequency of unique epitope sequences to generate the mosaic sequences. In this approach, the unique epitope sequences for each epitope are ordered by frequency in the alignment. For M vaccine proteins, the top M unique epitope sequences for each epitope site are included in the mosaic pattern.

The dominant epitopes mosaic sequences approach generates a set of mosaic patterns by taking the occurrence frequency ordered epitope sequences for layering into the mosaic sequence templates. The data flow and method steps are overviewed in FIG. 4. The step “dominant_epitopes1” takes as input the epitopes and columns files generated at the “find_epitopes_msa” step. An additional input specifies the ordered list of epitope names for the processing order of

epitopes to be added to the mosaic template sequences. This approach generates a FASTA formatted mosaic template file named “dominant”.

Embodiments of the present invention are based on the application of a methodology for the generation of mosaic B cell epitope sequences through structural mapping of antigenic repertoires. Vaccines developed to target specific viral isolates may not protect against infection from different isolates of the same virus. The approach for developing broadly protective antigens of the present invention, in some embodiments, creates polyvalent mosaic sequences that include B cell epitopes from as many viral isolates as possible. The individual sequences of known neutralizing antibody epitopes and antigenic sites are recombined to generate mosaic antigens. The best mosaic sequence templates are selected by evaluating overall alignment coverage by geographic regions, viral isolate years, sequence clusters or other scoring methods. The selected set of mosaic template patterns are combined with target backbone sequences to generate a set of full-length mosaic protein antigens. In some embodiments, structure refinement of these mosaic sequences yields the final set of vaccination proteins.

In some embodiments, the present invention is based on combinations of neutralizing and non-neutralizing hemagglutinin B cell epitope sequences derived from multiple influenza B type strains to generate novel mosaic designs for influenza hemagglutinin. Each design, consisting of a mosaic pattern of B-cell epitopes, is combined with a backbone hemagglutinin sequence. For example, backbone hemagglutinin sequences derived from B/Massachusetts/02/2012, B/Panama/45/1990, B/Singapore/222/79, or B/Brisbane/60/2008 are used in some embodiments. Other hemagglutinin backbone sequences may also be employed for construction of engineered HA polypeptides according to the present invention. In some embodiments, a backbone sequence provides the inter-epitope sequence as well as the signal peptide and transmembrane domains required for full-length hemagglutinin molecules that are expressed and functional.

In general, known neutralizing and non-neutralizing hemagglutinin B-cell epitopes described for any influenza B lineage strain are mapped to corresponding regions of the HA backbone. Individual sequences (antigenic repertoires) for each B cell epitopes are extracted and enumerated from the sequences of all available circulating influenza B strains. Distinct workflows, as described above, have been developed to combine the antigenic repertoires into novel mosaic hemagglutinin molecules that are distinct from natural circulating strains.

Exemplary workflows are further described in the Examples below. Although hemagglutinin polypeptides are used as examples to illustrate methods described herein, the present invention may be used to engineer neuraminidase (NA) polypeptides and other immunogenic polypeptides or therapeutic biologics.

Exemplary Mosaic Sequence Patterns and Engineered HA Polypeptides

The approaches described herein provide mosaic sequence patterns or consensus sequences that define overlapping or non-overlapping epitopes or antigenic regions. Desired epitopes or antigenic regions may be linear or discontinuous based on 3D structures including, but not limited to, antigenic regions (e.g., BA, BB1, BB2, BC, BD, BE, etc.) and/or antibody binding sites.

In some embodiments, the epitope or antigenic region is all or part of the Receptor-Binding Site (RBS). As used herein, the term “receptor-binding site” or “RBS” comprises

contiguous or non-contiguous amino acid residues of the head region of an influenza HA polypeptide, which include amino acids involved in direct binding of sialic acids on the target cell receptor proteins. The region of HA responsible for receptor binding resides at the membrane-distal tip of each monomer of the HA trimer, and it has several main structural features. For example, the binding site is flanked by the “220 and 130 loops”, which contain amino acids that interact with sialic acid or internal sugars of the glycan chain. The membrane-distal region of the site is formed by the 190 helix, which also includes residues with the potential to contact the receptor at either the sialic acid (residue 194) or internal glycans on the receptor (approximately residues 190 and 193). The base of the site contains several highly conserved residues that form an extensive hydrogen bond network. Amino acid residues that make up a “receptor-binding site” or “RBS” of an influenza HA polypeptide may be described from a three-dimensional crystal structures of HA polypeptides complexed with sialic acid analogs and identifying amino acid residues within a certain proximity to the analog or may be described in reference to an HA polypeptide sequence from a particular viral strain (e.g., A/New Caledonia/20/99 or A/California/07/2009). Thus, in some embodiments, the “receptor-binding site” or “RBS” of an engineered HA polypeptide as described herein may be determined using a reference HA polypeptide sequence. In some embodiments, the “receptor-binding site” or “RBS” of an engineered HA polypeptide as described herein may be determined using the crystal structures of HA polypeptide sequence in complex with human and avian receptor analogs (ex. LSTa, LSTc). An exemplary reference crystal structure of HA polypeptide sequence in complex with LSTc includes A/Puerto Rico/8/1934 (H1N1) pdb1RVZ. In some embodiments, the RBS may be defined as the epitope bound by the broadly neutralizing monoclonal antibody CH65 (see, e.g., Whittle J R, et al. Broadly neutralizing human antibody that recognizes the receptor-binding pocket of influenza virus hemagglutinin. *Proc Natl Acad Sci USA*. 2011; 108:14216-21). Alternatively or additionally, the RBS may be defined as an area including all amino acid residues within 15 Angstroms of a universally conserved tryptophan corresponding to position 167 in (CA09 09 Numbering) (e.g. see Xu, R et al. *Nat Struct Mol Biol*. 2013 March; 20(3):363-70) or corresponding position on a influenza type B virus HA polypeptide. An exemplary reference crystal structure of influenza type B HA polypeptide sequence includes B/Brisbane/60/2008 pdb14FQM. The corresponding tryptophan is residue 173 in the full-length sequence and 158 in the crystal structure 4FQM.

In various embodiments, an engineered HA polypeptide as described herein comprises an antigenic region that comprises contiguous or non-contiguous amino acid residues associated with, adjacent to, and/or encompass a receptor-binding site (RBS). In some embodiments, the non-contiguous amino acid residues can be determined using the crystal structures of HA polypeptide. An exemplary reference crystal structure of HA polypeptide sequence includes B/Yamanashi/166/1998 pdb14M40.

In some embodiments, an RBS site comprises a mosaic sequence pattern that comprises amino acid substitutions at residues: 110, 140, 141, 143, 145, 146, 153, 154, 155, 156, 165, 174, 175, 176, 177, 179, 180, 181, 209, 210, 211, 212, 215, 216, 217, 228, 258, 259, and/or 279 or a subset thereof, (e.g., with at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, or 10 referenced residues), as indexed by reference to a B/Brisbane/60/2008 amino acid sequence.

In some embodiments, an RBS site comprises a mosaic sequence pattern that comprises amino acid substitutions at residues: 110F, 140I, 141D/N, 143E, 145A, 146P, 153G, 154T, 155S, 156G/R, 165S/I/N, 174A, 175V, 176P, 177K, 179D, 180N/S, 181N, 209H, 210S, 211D, 212N/D, 215Q, 216M, 217K/V, 228F, 258S, 259G, and/or 279Q, or a subset thereof, (e.g., with at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, or 10 referenced residues), as indexed by reference to a B/Brisbane/60/2008 amino acid sequence.

In some embodiments, a mosaic sequence pattern comprises amino acid substitutions at residues: 21, 22, 23, 26, 37, 38, 43, 45, 46, 47, 52, 53, 54, 55, 56, 58, 60, 62, 63, 67, 70, 73, 74, 75, 76, 77, 85, 86, 89, 90, 91, 92, 93, 94, 95, 100, 101, 102, 103, 104, 105, and/or 106, or a subset thereof (e.g., with at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, or 10 referenced residues), as indexed by reference to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern that comprises amino acid residues: 21G, 22I, 23T, 26N/K, 37G, 38E, 43G, 45I, 46P/S, 47L, 52T, 53K, 54S, 55Y/H, 56F, 58N, 60K/R, 62T, 63E/K, 67K, 70P, 73L/P, 74N, 75C, 76T, 77D, 85P, 86K/M, 89G, 90K/T/N, 91I, 92P, 93S, 94A, 95R/K, 100H, 101E, 102V, 103R, 104P, 105V, and/or 106T, or a subset thereof (e.g., with at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, or 10 referenced residues), as indexed by reference to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern that comprises amino acid substitutions at residues: 128, 130, 131, 133, 136, 137, 138, 159, 160, 161, 162, 165, 173, 195, 196, 197, 198, 199, 200, 213, 214, 225, 227, 245, 248, 250, 251, 257, and/or 277 or a subset thereof (e.g., with at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, or 10 referenced residues), as indexed by reference to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern that comprises amino acid residues: 128G, 130E, 131H, 133R, 136T/N, 137Q/H/Y, 138N, 159P, 160N, 161A/V, 162T, 165K/R, 173W, 195I, 196C, 197T/A, 198E, 199G, 200E, 213K, 214T, 225P, 227K, 245G/D, 248D/N, 250T, 251E, 257Q, and/or 277V/T or a subset thereof (e.g., with at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, or 10 referenced residues), as indexed by reference to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern that comprises amino acid residues: 290, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 314, 315, 319, 320, 321, 322, 323, 348, 354, 355, 356, 377, 378, 380, 381, 382, 383, 387, 392, 394, 395, 396, 397, 398, 400, 401, 403, 404, 405, 406, 407, 408, 410, 411, 412, 413, 414, 415, 417, 418, 419, 420, 421, 422, 470, 508, and/or 512, or a subset thereof (e.g., with at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, or 10 referenced residues), as indexed by reference to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern that comprises amino acid substitutions at residues: 290C, 300G, 301S/T, 302L, 303P, 304L, 305I, 306G, 307E, 308A, 309D, 314K, 315Y, 319N, 320K, 321S, 322K, 323P, 348N, 354P, 355P, 356A/T, 377E, 378G, 380V/I, 381A, 382G, 383W, 387T, 392H, 394V, 395A, 396V, 397A, 398A, 400L, 401K, 403T, 404Q, 405E, 406A, 407I, 408N, 410I, 411T, 412K,

31

413N, 414L, 415N, 417L, 418S, 419E, 420L, 421E, 422I, 470I, 508Q, and/or 512D/N, or a subset thereof, (e.g., with at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 referenced residues), as indexed by reference

to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern or consensus sequence according to the invention comprises residues: 110F, 140I, 141N, 143E, 145A, 146P, 153G, 154T, 155S, 156G, 165S, 174A, 175V, 176P, 177K, 179D, 180N, 181N, 209H, 210S, 211D, 212N, 215Q, 216M, 217K, 228F, 258S, 259G, 279Q, 21G, 22I, 23T, 26N, 37G, 38E, 43G, 45I, 46P, 47L, 52T, 53K, 54S, 55H, 56F, 58N, 60K, 62T, 63E, 67K, 70P, 73L, 74N, 75C, 76T, 77D, 85P, 86K, 89G, 90K, 91I, 92P, 93S, 94A, 95R, 100H, 101E, 102V, 103R, 104P, 105V, 106T, 128G, 130E, 131H, 133R, 136T, 137Q, 138N, 159P, 160N, 161A, 162T, 165K, 173W, 195I, 196C, 197T, 198E, 199G, 200E, 213K, 214T, 225P, 227K, 245G, 248N, 250T, 251E, 257Q, 277V, 290C, 300G, 301S, 302L, 303P, 304L, 305I, 306G, 307E, 308A, 309D, 314K, 315Y, 319N, 320K, 321S, 322K, 323P, 348N, 354P, 355P, 356A, 377E, 378G, 380I, 381A, 382G, 383W, 387T, 392H, 394V, 395A, 396V, 397A, 398A, 400L, 401K, 403T, 404Q, 405E, 406A, 407I, 408N, 410I, 411T, 412K, 413N, 414L, 415N, 417L, 418S, 419E, 420L, 421E, 422V, 470I, 508Q, and/or 512D, or a subset thereof (e.g., with at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 referenced residues), as indexed by reference to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern or consensus sequence according to the invention comprises residues: 110F, 140I, 141D, 143E, 145A, 146P, 153G, 154T, 155S, 156G, 165S, 174A, 175V, 176P, 177K, 179D, 180N, 181N, 209H, 210S, 211D, 212N, 215Q, 216M, 217K, 228F, 258S, 259G, 279Q, 21G, 22I, 23T, 26N, 37G, 38E, 43G, 45I, 46P, 47L, 52T, 53K, 54S, 55Y, 56F, 58N, 60K, 62T, 63E, 67K, 70P, 73L, 74N, 75C, 76T, 77D, 85P, 86K, 89G, 90K, 91I, 92P, 93S, 94A, 95K, 100H, 101E, 102V, 103R, 104P, 105V, 106T, 128G, 130E, 131H, 133R, 136T, 137Q, 138N, 159P, 160N, 161A, 162T, 165K, 173W, 195I, 196C, 197T, 198E, 199G, 200E, 213K, 214T, 225P, 227K, 245G, 248N, 250T, 251E, 257Q, 277V, 290C, 300G, 301S, 302L, 303P, 304L, 305I, 306G, 307E, 308A, 309D, 314K, 315Y, 319N, 320K, 321S, 322K, 323P, 348N, 354P, 355P, 356A, 377E, 378G, 380V, 381A, 382G, 383W, 387T, 392H, 394V, 395A, 396V, 397A, 398A, 400L, 401K, 403T, 404Q, 405E, 406A, 407I, 408N, 410I, 411T, 412K, 413N, 414L, 415N, 417L, 418S, 419E, 420L, 421E, 422I, 470I, 508Q, and/or 512D, or a subset thereof or a subset thereof (e.g., with at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 referenced residues), as indexed by reference to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern or consensus sequence according to the invention comprises residues: 110F, 140I, 141D, 143E, 145A, 146P, 153G, 154T, 155S, 156G, 165S, 174A, 175V, 176P, 177K, 179D, 180N, 181N, 209H, 210S, 211D, 212N, 215Q, 216M, 217K, 228F, 258S, 259G, 279Q, 21G, 22I, 23T, 26N, 37G, 38E, 43G, 45I, 46P, 47L, 52T, 53K, 54S, 55Y, 56F, 58N, 60K, 62T, 63E, 67K, 70P, 73L, 74N, 75C, 76T, 77D, 85P, 86K, 89G, 90K, 91I, 92P, 93S, 94A, 95K, 100H, 101E, 102V, 103R, 104P, 105V, 106T, 128G, 130E, 131H, 133R, 136T, 137Q, 138N, 159P, 160N, 161A, 162T, 165K, 173W, 195I, 196C,

32

197T, 198E, 199G, 200E, 213K, 214T, 225P, 227K, 245G, 248N, 250T, 251E, 257Q, 277V, 290C, 300G, 301S, 302L, 303P, 304L, 305I, 306G, 307E, 308A, 309D, 314K, 315Y, 319N, 320K, 321S, 322K, 323P, 348N, 354P, 355P, 356A, 377E, 378G, 380V, 381A, 382G, 383W, 387T, 392H, 394V, 395A, 396V, 397A, 398A, 400L, 401K, 403T, 404Q, 405E, 406A, 407I, 408N, 410I, 411T, 412K, 413N, 414L, 415N, 417L, 418S, 419E, 420L, 421E, 422I, 470I, 508Q, and/or 512D, or a subset thereof or a subset thereof (e.g., with at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 referenced residues), as indexed by reference to a B/Brisbane/60/2008 HA amino acid sequence.

In some embodiments, a mosaic sequence pattern or consensus sequence according to the invention comprises residues: 110F, 140I, 141D, 143E, 145A, 146P, 153G, 154T, 155S, 156R, 165S, 174A, 175V, 176P, 177K, 179D, 180S, 181N, 209H, 210S, 211D, 212N, 215Q, 216M, 217V, 228F, 258S, 259G, 279Q, 21G, 22I, 23T, 26N, 37G, 38E, 43G, 45I, 46P, 47L, 52T, 53K, 54S, 55H, 56F, 58N, 60R, 62T, 63K, 67K, 70P, 73L, 74N, 75C, 76T, 77D, 85P, 86K, 89G, 90N, 91T, 92P, 93S, 94A, 95K, 100H, 101E, 102V, 103R, 104P, 105V, 106T, 128G, 130E, 131H, 133R, 136N, 137Y, 138N, 159P, 160N, 161V, 162T, 165R, 173W, 195I, 196C, 197T, 198E, 199G, 200E, 213K, 214T, 225P, 227K, 245D, 248N, 250T, 251E, 257Q, 277T, 290C, 300G, 301T, 302L, 303P, 304L, 305I, 306G, 307E, 308, 309D, 314K, 315Y, 319N, 320K, 321S, 322K, 323P, 348N, 354P, 355P, 356A, 377E, 378G, 380I, 381A, 382G, 383W, 387T, 392H, 394V, 395A, 396V, 397A, 398A, 400L, 401K, 403T, 404Q, 405E, 406A, 407I, 408N, 410I, 411T, 412K, 413N, 414L, 415N, 417L, 418S, 419E, 420L, 421E, 422V, 470I, 508Q, and/or 512D, or a subset thereof (e.g., with at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 referenced residues), as indexed by reference to a B/Panama/45/1990 HA amino acid sequence.

In some embodiments, an engineered HA polypeptide of the present invention comprises an antigenic region that comprises amino acid residues 110F, 140I, 141N, 143E, 145A, 146P, 153G, 154T, 155S, 156G, 165N, 174A, 175V, 176P, 177K, 179D, 180N, 181N, 209H, 210S, 211D, 212N, 215Q, 216M, 217K, 228F, 258S, 259G, 279Q, 21G, 22I, 23T, 26N, 37G, 38E, 43G, 45I, 46P, 47L, 52T, 53K, 54S, 55Y, 56F, 58N, 60K, 62T, 63K, 67K, 70P, 73L, 74N, 75C, 76T, 77D, 85P, 86M, 89G, 90T, 91I, 92P, 93S, 94A, 95K, 100H, 101E, 102V, 103R, 104P, 105V, 106T, 128G, 130E, 131N, 133R, 136T, 137H, 138N, 159P, 160N, 161A, 162T, 165K, 173W, 195I, 196C, 197T, 198E, 199G, 200E, 213K, 214T, 225P, 227K, 245G, 248D, 250T, 251E, 257Q, 277T, 290C, 300G, 301S, 302L, 303P, 304L, 305I, 306G, 307E, 308A, 309D, 314K, 315Y, 319N, 320K, 321S, 322K, 323P, 348N, 354P, 355P, 356A, 377E, 378G, 380I, 381A, 382G, 383W, 387T, 392H, 394V, 395A, 396V, 397A, 398A, 400L, 401K, 403T, 404Q, 405E, 406A, 407I, 408N, 410I, 411T, 412K, 413N, 414L, 415N, 417L, 418S, 419E, 420L, 421E, 422V, 470I, 508Q, and/or 512D, or a subset thereof (e.g., with at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% of the referenced residues, or at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 referenced residues), as indexed by reference to a, B/Massachusetts/02/2012 amino acid sequence.

Exemplary mosaic amino acid sequence patterns based on different SMART workflows (e.g., SMART dominant (DO),

SMART Random (RA), SMART Collapse (CO), or SMART Choice (CI)) are provided in Table 1.

TABLE 1					
	DO2	DO3	CO1	RA82	RA103
21	G	G	G	G	G
22	I	I	I	I	I
23	T	T	T	T	T
26	N	N	N	K	N
37	G	G	G	G	G
38	E	E	E	E	E
43	G	G	G	G	G
45	I	I	I	I	I
46	P	S	P	P	P
47	L	L	L	L	L
52	T	T	T	T	T
53	K	K	K	K	K
54	S	S	S	S	S
55	Y	H	H	H	Y
56	F	F	F	F	F
58	N	N	N	N	N
60	K	K	K	R	K
62	T	T	T	T	T
63	E	K	E	K	K
67	K	K	K	K	K
70	P	P	P	P	P
73	L	P	L	L	L
74	N	N	N	N	N
75	C	C	C	C	C
76	T	T	T	T	T
77	D	D	D	D	D
85	P	P	P	P	P
86	K	M	K	K	M
89	G	G	G	G	G
90	K	T	K	N	T
91	I	I	I	T	I
92	P	P	P	P	P
93	S	S	S	S	S
94	A	A	A	A	A
95	K	K	R	K	K
100	H	H	H	H	H
101	E	E	E	E	E
102	V	V	V	V	V
103	R	R	R	R	R
104	P	P	P	P	P
105	V	V	V	V	V
106	T	T	T	T	T
110	F	F	F	F	F
128	G	G	G	G	G
130	E	E	E	E	E
131	H	H	H	H	N
133	R	R	R	R	R
136	T	T	T	N	T
137	Q	H	Q	Y	H
138	N	N	N	N	N
140	I	I	I	I	I
141	D	N	N	D	N
143	E	E	E	E	E
145	A	A	A	A	A
146	P	P	P	P	P
153	G	G	G	G	G
154	T	T	T	T	T
155	S	S	S	S	S
156	G	G	G	R	G
159	P	P	P	P	P
160	N	N	N	N	N
161	A	A	A	V	A
162	T	T	T	T	T
165	S	I	S	S	N
165	K	K	K	R	K
173	W	W	W	W	W
174	A	A	A	A	A
175	V	V	V	V	V
176	P	P	P	P	P
177	K	K	K	K	K
179	D	D	D	D	D
180	N	N	N	S	N
181	N	N	N	N	N
195	I	I	I	I	I

TABLE 1-continued

	DO2	DO3	CO1	RA82	RA103
196	C	C	C	C	C
197	T	A	T	T	T
198	E	E	E	E	E
199	G	G	G	G	G
200	E	E	E	E	E
209	H	H	H	H	H
210	S	S	S	S	S
211	D	D	D	D	D
212	N	D	N	N	N
213	K	K	K	K	K
214	T	T	T	T	T
215	Q	Q	Q	Q	Q
216	M	M	M	M	M
217	K	K	K	V	K
225	P	P	P	P	P
227	K	K	K	K	K
228	F	F	F	F	F
245	G	D	G	D	G
248	D	N	N	N	D
250	T	T	T	T	T
251	E	E	E	E	E
257	Q	Q	Q	Q	Q
258	S	S	S	S	S
259	G	G	G	G	G
277	V	T	V	T	T
279	Q	Q	Q	Q	Q
290	C	C	C	C	C
300	G	G	G	G	G
301	S	S	S	T	S
302	L	L	L	L	L
303	P	P	P	P	P
304	L	L	L	L	L
305	I	I	I	I	I
306	G	G	G	G	G
307	E	E	E	E	E
308	A	A	A	—	A
309	D	D	D	D	D
314	K	K	K	K	K
315	Y	Y	Y	Y	Y
319	N	N	N	N	N
320	K	K	K	K	K
321	S	S	S	S	S
322	K	K	K	K	K
323	P	P	P	P	P
348	N	N	N	N	N
354	P	P	P	P	P
355	P	P	P	P	P
356	A	T	A	A	A
377	E	E	E	E	E
378	G	G	G	G	G
380	V	I	I	I	I
381	A	A	A	A	A
382	G	G	G	G	G
383	W	W	W	W	W
387	T	T	T	T	T
392	H	H	H	H	H
394	V	V	V	V	V
395	A	A	A	A	A
396	V	V	V	V	V
397	A	A	A	A	A
398	A	A	A	A	A
400	L	L	L	L	L
401	K	K	K	K	K
403	T	T	T	T	T
404	Q	Q	Q	Q	Q
405	E	E	E	E	E
406	A	A	A	A	A
407	I	I	I	I	I
408	N	N	N	N	N
410	I	I	I	I	I
411	T	T	T	T	T
412	K	K	K	K	K
413	N	N	N	N	N
414	L	L	L	L	L
415	N	N	N	N	N
417	L	L	L	L	L
418	S	S	S	S	S
419	E	E	E	E	E

TABLE 1-continued

	DO2	DO3	CO1	RA82	RA103
420	L	L	L	L	L
421	E	E	E	E	E
422	I	V	V	V	V
470	I	I	I	I	I
508	Q	Q	Q	Q	Q
512	D	N	D	D	D

As non-limiting examples, full-length mosaic sequence patterns or consensus sequence templates are shown on Table 2. “-” shown in the templates on Table 2 stands for any amino acid or a peptide bond. The templates may be subsequently merged onto a backbone sequence to provide the missing sequence information.

Selected templates of mosaic sequence patterns or consensus sequences may be inserted into corresponding locations of a structural backbone of HA or NA to generate mosaic influenza HA or NA polypeptide. For example, one or more amino acid substitutions according to mosaic sequence patterns as shown in Table 1 may be inserted in various corresponding locations in a backbone sequence. In some embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the referenced residues as shown in Table 1 are inserted in corresponding locations in a backbone sequence. In some embodiments, at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 referenced residues shown in Table 1 are inserted in corresponding locations in a backbone sequence.

TABLE 2

DO2_template	-----GIT--N-----GE---G-IPL----TKSYF-N-K-TE--- K--P--LNCTD-----PK--GKIPSAK---HEVRPVT---F----- G-EH-R--TQN-ID-E-AP-----GTSG--PNAT-KS-----WAVPK- DNN-----ICTEGE-----HSDNKTQMK-----P-KF----- ---G--D-TE-----QSG-----KPG-----V-Q-----C----- GSLPLIGEAD---KY---NKSKP-----N---PPA----- -----EG-VAGW---T---H-VAVAA-LK-TQEAIN-ITKNLN- LSELEI-----I----- -----Q---D (SEQ ID NO: 1)
DO3_template	-----GIT--N-----GE---G-ISL----TKSHF-N-K-TK--- K--P--PNCTD-----PM--GTIPSAK---HEVRPVT---F----- G-EH-R--THN-IN-E-AP-----GTSG--PNAT-KI-----WAVPK-DNN- -----ICAEGE-----HSDDKTQMK-----P-KF-----D--- N-TE-----QSG-----KPG-----T-Q-----C-----GSLPLIGEAD-- --KY---NKSKP-----N---PPT-----EG- IAGW---T---H-VAVAA-LK-TQEAIN-ITKNLN-LSELEV----- -----I-----Q---N (SEQ ID NO: 2)
CO1_template	-----GIT--n-----GE---G-IPL----TKShF-N-K-Te---K-- P--lNCTD-----Pk--GkiPSAr---HEVRPVT---F-----G-Eh- R--TqN-In-E-AP-----GTSG--PNat-ks-----WAVPK-DnN----- -ICtEGE-----HSDnktQmk-----P-KF-----g--n-TE----- QSG-----KpG-----v-Q-----C-----GSLPLIGEAD---KY--- NKSKP-----N---PPA-----EG-IAGW--- T---H-VAVAA-LK-TQEAIN-ITKNLN-LSELEV----- -----I-----Q---D (SEQ ID NO: 3)
RA82_template	-----GIT--K-----GE---G-IPL----TKSHF-N-R-TK--- K--P--LNCTD-----PK--GNTPSAK---HEVRPVT---F----- G-EH-R--NYN-ID-E-AP-----GTSR--PNVT-RS-----WAVPK- DSN-----ICTEGE-----HSDNKTQMV-----P-KF----- --D--N-TE-----QSG-----KSG-----T-Q-----C----- GTLPLIGE-D---KY---NKSKP-----N---PPA----- -----EG-IAGW---T---H-VAVAA-LK-TQEAIN-ITKNLN- LSELEV-----I----- -----Q---D (SEQ ID NO: 4)
RA103_template	-----GIT--N-----GE---G-IPL----TKSYF-N-K-TK--- K--P--LNCTD-----PM--GTIPSAK---HEVRPVT---F----- G-EN-R--THN-IN-E-AP-----GTSG--PNAT-KN-----WAVPK- DNN-----ICTEGE-----HSDNKTQMK-----P-KF----- ---G--D-TE-----QSG-----KSG-----T-Q-----C----- GSLPLIGEAD---KY---NKSKP-----N---PPA----- -----EG-IAGW---T---H-VAVAA-LK-TQEAIN-ITKNLN- LSELEV-----I----- -----Q---D (SEQ ID NO: 5)

A suitable structural backbone may be derived from different lineages. For example, a suitable structural backbone may be derived from a Yamagata lineage or a victoria lineage. In the some embodiments, one of the following nine backbone hemagglutinin sequences may be used: B/Hong Kong/330/2001, B/Hong Kong/05/1972, B/Lee/40, B/Massachusetts/02/2012, B/Panama/45/1990, B/Singapore/222/79, B/Victoria/02/1987, B/Yamagata/16/1988, B/Brisbane/60/2008.

Exemplary backbone sequences are shown in Table 3.

TABLE 3

60780 CAA25425 HA Human fluB B/Singapore/222/ 79 Singapore 1979	DRICTGITSSNSPHVVKATATQGEVNVTVGVPLTTTPTKSHFANL KGTCTRGKLCPCNCLNCTDLVALGRPKCMGTIPSAKASILHEV KPVTSKGCFFIMHDKIRQLPNLLRGYENIRLSTRNVINAERAP GGPYIIGTSGSCPNVTNGNGHATMAWAVPKD - NKTATNPLTVEVPYICTKGEDQITVWGFHSDTETQMVKLYGD SKPQKFTSSANGVTTHYVSQIGGFNPQTEDGGLPQSGRIVVDY MVQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSPLIGE ADCLHEKYGGLNKSPPYTGEGHAKAIGNCPIWVKTPKLKANG TKYRPPAKLLKERGFAGAIAGFLEGGWEGMIAGWHGYTSHGA HGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMD ELHNEILELDEKVDDLADTISQIELAVLLSNEGIINSEDEHLL ALERKLLKMLGPSAVDIGNGCFETKHKNQTCLDRIAAGTFN AGEFSLPTFDSLNTAASLNDDGLDNHTILLYYSTAASSLAVTL MIAIFIVYMVRDNVSCSICL (SEQ ID NO: 6)
488466015 AGL06036 HA Human fluB B/Massachusetts/ 02/2012 USA 2012/03/13	DRICTGITSSNSPHVVKATATQGEVNVTVGVPLTTTPTKSYFANL KGTCTRGKLCPCDCLNCTDLVALGRPMCIVGTTPSAKASILHE VRPVTSGCFFIMHDKIRQLANLLRGYENIRLSTQNVDAEKA PGGPYRLGTSGSCPNATSKSGFFATMAWAVPKD - NNKNATNPLTVEVPYICAEGEDQITVWGFHSDDKTQMKNLGY DSNPQKFTSSANGVTTHYVSQIGGFNPQTEDGGLPQSGRIVVD YMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSPLIG EADCLHEKYGGLNKSPPYTGEGHAKAIGNCPIWVKTPKLKANG GTYRPPAKLLKERGFAGAIAGFLEGGWEGMIAGWHGYTSHG AHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAM DELHNEILELDEKVDDLADTISQIELAVLLSNEGIINSEDEHL LALERKLLKMLGPSAVDIGNGCFETKHKNQTCLDRIAAGTF NAGEFSLPTFDSLNTAASLNDDGLDNHTILLYYSTAASSLAVT LMLAIFIVYMVRDNVSCSICL (SEQ ID NO: 7)
119515731 ABL76694 HA Human fluB B/Panama/45/ 1990 Panama 1990/03/07	DRICTGITSSNSPHVVKATATQGEVNVTVGVPLTTTPTKSHFANL KGTCTRGKLCPCNCLNCTDLVALGRPMCIVGTTPSAKASILHE VRPVTSGCFFIMHDKIRQLPNLLRGYENIRLSTQNVINAERA PGGPYRLGTSGSCPNVTSRDGFFATMAWAVPRD - - NKTATNPLTVEVPYICTKGEDQITVWGFHSDNKTQMKNLGYD SNPQKFTSSANGVTTHYVSQIGGFNPQTEDGGLPQSGRIVVDY MVQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSPLIGE ADCLHEKYGGLNKSPPYTGEGHAKAIGNCPIWVKTPKLKANG TKYRPPAKLLKERGFAGAIAGFLEGGWEGMIAGWHGYTSHGA HGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMD ELHNEILELDEKVDDLADTISQIELAVLLSNEGIINSEDEHLL ALERKLLKMLGPSAVDIGNGCFETKHKNQTCLDRIAAGTFN AGEFSLPTFDSLNTAASLNDDGLDNHTILLYYSTAASSLAVTL MIAIFIVYMVRDNVSCSICL (SEQ ID NO: 8)
384038646 AFH57909 HA Human fluB B/Brisbane/ 60/2008 Australia 2008	DRICTGITSSNSPHVVKATATQGEVNVTVGVPLTTTPTKSHFANL KGTETRGKLCPCCLNCTDLVALGRPKCTGKIPSARVSILHEV RPVTSKGCFFIMHDKIRQLPNLLRGYEHIRLSTHNVINAENAP GGPYKIGTSGSCPNITNGNGIMATMAWAVPKNDKNKTATNPL TIEVPYICTEGEDQITVWGFHSDDETQMAKLYGDSKPQKFTSS ANGVTTHYVSQIGGFNPQTEDGGLPQSGRIVVDYMVQKSGKT GTITYQRGILLPQKVWCASGRSKVIKGSPLIGEGADCLHEKY GLNKSPPYTGEGHAKAIGNCPIWVKTPKLKANGTKYRPPAKL LKERGFAGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAAD LKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELD EKVDDLADTISQIELAVLLSNEGIINSEDEHLLALERKLLKML LGPSAVEIGNGCFETKHKNQTCLDRIAAGTFDAGEFSLPTFDS LNITAASLNDDGLDNHTILLYYSTAASSLAVTLMIAIFVYVMV SRDNVSCSICL (SEQ ID NO: 9)

In various embodiments, engineered HA polypeptides as described herein comprise combinations of epitope sequences merged onto a particular viral backbone sequence. Multiple epitopes can be assembled on to any viral backbone as desired. In some embodiments, engineered HA polypeptides as described herein comprise a B/Massachusetts/02/2012, B/Panama/45/1990, B/Singapore/222/79, or B/Brisbane/60/2008 backbone sequence. Exemplary engineered mosaic HA polypeptides of the present invention are shown in Table 4.

TABLE 4

br08_CO1	MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTAT QGEVNVTVGIPLTTTTPTKSHFANLKGTTETRGKLC KCLNCTDLVALGRPKCTGKIP SARVSILHEVRPV TSGCFPIIMHDRTKIRQLPNLLRGYEHIRLSTQNVIN AENAPGGPYKIGTSGSCPNATNKSGFFATMAWAV PKNDNNKTATNPLTIEVPYICTEGEDQITVWGFHS DNKTQMKKLYGDSKPQKFTSSANGVTTHYVSQIG GFPNQTEDGGLPQSGRIVVDYMVQKPGKTGTIVY QRGILLPQKVWCASGRSKVIKGSPLPLIGEADCLHE KYGGLNKS KPYYTGEHAKAIGNCPIWVKTP LKLA NGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIA GWHGYTSHGAHGVAVAADLKSTQEAINKITKNL NSLSELEVKNLQRLSGAMDELHNEILELDEKVDD LRADTISSQIELAVLLSNEGIINSEDEHLLALERKL KKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGT IDAGEFSLPTFDSLNI TAASLNDDGLDNHTILLYYS TAASSLAVTLMIAIFVVYMVSRDNVSCSICL (SEQ ID NO: 40)	5
br08_DO2	MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTAT QGEVNVTVGIPLTTTTPTKSYFANLKGTTETRGKLC KCLNCTDLVALGRPKCTGKIPSAKVSILHEVRPV TSGCFPIIMHDRTKIRQLPNLLRGYEHIRLSTQNVID AENAPGGPYKIGTSGSCPNATNKSGFFATMAWAV PKNDNNKTATNPLTIEVPYICTEGEDQITVWGFHS DNKTQMKKLYGDSKPQKFTSSANGVTTHYVSQIG GFPDQTEDGGLPQSGRIVVDYMVQKPGKTGTIVY QRGILLPQKVWCASGRSKVIKGSPLPLIGEADCLHE KYGGLNKS KPYYTGEHAKAIGNCPIWVKTP LKLA NGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMV AGWHGYTSHGAHGVAVAADLKSTQEAINKITKN LNSLSELEIKNLQRLSGAMDELHNEILELDEKVDD LRADTISSQIELAVLLSNEGIINSEDEHLLALERKL KKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGT FDAGEFSLPTFDSLNI TAASLNDDGLDNHTILLYYS TAASSLAVTLMIAIFVVYMVSRDNVSCSICL (SEQ ID NO: 41)	20
br08_DO3	MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTAT QGEVNVTVGISLTTTTPTKSHFANLKGTTKTRGKLC KCPNCTDLVALGRPMC GTIPSAKVSILHEVRPV TSGCFPIIMHDRTKIRQLPNLLRGYEHIRLSTHNVIN AENAPGGPYKIGTSGSCPNATNKIGFFATMAWAV PKNDNNKTATNPLTIEVPYICAEGEDQITVWGFHS DDKTQMKKLYGDSKPQKFTSSANGVTTHYVSQIG DFPNQTEDGGLPQSGRIVVDYMVQKPGKTGTITY QRGILLPQKVWCASGRSKVIKGSPLPLIGEADCLHE KYGGLNKS KPYYTGEHAKAIGNCPIWVKTP LKLA NGTKYRPPTKLLKERGFFGAIAGFLEGGWEGMIA GWHGYTSHGAHGVAVAADLKSTQEAINKITKNL NSLSELEVKNLQRLSGAMDELHNEILELDEKVDD LRADTISSQIELAVLLSNEGIINSEDEHLLALERKL KKMLGPSAVEIGNGCFETKHKCNQTCLNRIAAGT FDAGEFSLPTFDSLNI TAASLNDDGLDNHTILLYYS TAASSLAVTLMIAIFVVYMVSRDNVSCSICL (SEQ ID NO: 42)	35
pan90_DO2	MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTAT QGEVNVTVGIPLTTTTPTKSYFANLKGTTETRGKLC NCLNCTDLVALGRPKCVGKIPSAKASILHEVRPV TSGCFPIIMHDRTKIRQLPNLLRGYEHIRLSTQNVID AERAPGGPYRLGTSGSCPNATSKSGFFATMAWAV PKDDNNKTATNPLTVEVPYICTEGEDQITVWGFH SDNKTQMKNLYGDSNPQKFTSSANGVTTHYVSQI GGFPDQTEDGGLPQSGRIVVDYMVQKPGKTGTIV YQRGVLLPQKVWCASGRSKVIKGSPLPLIGEADCL HEKYGGLNKS KPYYTGEHAKAIGNCPIWVKTP LK LANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGM VAGWHGYTSHGAHGVAVAADLKSTQEAINKITK NLNSLSELEIKNLQRLSGAMDELHNEILELDEKVD DLRADTISSQIELAVLLSNEGIINSEDEHLLALERK LKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAG TFNAGEFSLPTFDSLNI TAASLNDDGLDNHTILLY YSTAASSLAVTLMIAIFIVYMVSRDNVSCSICL (SEQ ID NO: 43)	50
ma12_RA82	MKAIIVLLMVVTSNADRICTGITSSKSPHVVKTAT QGEVNVTVGIPLTTTTPTKSHFANLRGTTKTRGKLC DCLNCTDLVALGRPKCVGNTPSAKASILHEVRP VTSGCFPIIMHDRTKIRQLANLLRGYEHIRLSNYN	65

TABLE 4-continued

	IDAEKAPGGPYRLGTSRSCPNVTSRSGFFATMAW AVPKDDSNKNATNPLTVEVPYICTEGEDQITVWG FHSDNKTQMVNLYGDSNPQKFTSSANGVTTHYV SQIGDFPNQTEDGGLPQSGRIVVDYMMQKSGKTG TITYQRGVLLPQKVWCASGRSKVIKGTLP LIG CLHEKYGGLNKS KPYYTGEHAKAIGNCPIWVKTP LKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWE GMIAGWHGYTSHGAHGVAVAADLKSTQEAINKI TKNLNSLSELEVKNLQRLSGAMDELHNEILELDE KVDDL RADTISSQIELAVLLSNEGIINSEDEHLLAL ERK LKKMLGPSAVDIGNGCFETKHKCNQTCLDRI AAGTFNAGEFSLPTFDSLNI TAASLNDDGLDNHTI LLYYSTAASSLAVTLMIAIFIVYMVSRDNVSCSIC L (SEQ ID NO: 44)	
sing79_RA103	MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTAT QGEVNVTVGIPLTTTTPTKSYFANLKGTTKTRGKLC NCLNCTDLVALGRPMC GTIPSAKASILHEVRP VTSGCFPIIMHDRTKIRQLPNLLRGYENIRLSTHNVI NAERAPGGPYIIGTSGSCPNATNKNGFFATMAWA VPKDDNNKTATNPLTVEVPYICTEGEDQITVWGF HSDNKTQMKKLYGDSKPQKFTSSANGVTTHYVS QIGGFPDQTEDGGLPQSGRIVVDYMVQKSGKTGT ITYQRGVLLPQKVWCASGRSKVIKGSPLPLIG ADCLHEKYGGLNKS KPYYTGEHAKAIGNCPIWVKTP LKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEG MIAGWHGYTSHGAHGVAVAADLKSTQEAINKIT KNLNSLSELEVKNLQRLSGAMDELHNEILELDEK VDDL RADTISSQIELAVLLSNEGIINSEDEHLLALE RKLKMLGPSAVDIGNGCFETKHKCNQTCLDRIA AGTFNAGEFSLPTFDSLNI TAASLNDDGLDNHTIL LYYSTAASSLAVTLMIAIFIVYMVSRDNVSCSICL (SEQ ID NO: 45)	

In some embodiments, an engineered HA polypeptide of the present invention has a sequence at least about 95% (e.g., at least about 96%, 97%, 98% or 99%) identical to a sequence that appears in Table 4, wherein the sequence is not a naturally-occurring sequence. In some embodiments, an engineered HA polypeptide of the present invention has a sequence at least about 95% (e.g., at least about 96%, 97%, 98% or 99%) identical to a sequence that appears in Table 4, and further comprises a mosaic sequence pattern or consensus sequence template shown on Table 2.

In some embodiments, an engineered HA polypeptide of the present invention has a sequence that is substantially identical to a sequence that appears in Table 4.

In some embodiments, an engineered HA polypeptide of the present invention has a sequence that is identical to a sequence that appears in Table 4.

In some embodiments, an engineered HA polypeptide of the present invention is selected from a sequence that appears in Table 4.

In some embodiments, mosaic antigens as described herein are refined by molecular modeling to resolve structural problems and generate energy-minimized designs for engineered HA polypeptides. The total energies of the resulting designed molecules are calculated using a design algorithm. For example, the design algorithm in the Rosetta molecular modeling software version 3.1 (Simons et al., 1997, J. Mol. Biol. 268:209-225; Leaver-Fay et al., 2011, Methods Enzymol. 487:545-574) may be used. Molecules with negative total energy scores are predicted to have a good probability of folding into stable proteins while those with positive energy scores are considered less likely to fold properly. In addition to the total energy scores, per-residue energy scores for each molecule are evaluated in order to identify hot-spots (i.e., specific amino acid residues in the designed structures that had positive energy scores). Such hot spots may induce local misfolding with negative impact

on epitope presentation on the surface of the molecule. The modeled structures are carefully examined visually to determine the location of the hot-spot residues relative to the surface of the molecule. In some embodiments, to further stabilize the molecular designs, high-energy residues situated outside of important epitope regions are substituted to more stable alternatives compatible with the local structural context. The resulting designs are more stable than the initial versions as determined by recalculated total energy scores.

Nucleic Acid Construction and Expression

Engineered influenza B HA or NA polypeptides as described herein may be produced from nucleic acid molecules using molecular biological methods known to the art. Nucleic acid molecules are inserted into a vector that is able to express the HA or NA polypeptides when introduced into an appropriate host cell. Appropriate host cells include, but are not limited to, bacterial, yeast, insect, and mammalian cells. Nucleic acid sequences may be codon optimized to facilitate expression in any of these host cells. Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the fusion proteins of the present invention under control of transcriptional/translational control signals. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombination (See Sambrook et al. *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory; Current Protocols in Molecular Biology, Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY).

In some embodiments, the present invention provides nucleic acids which encode an HA or NA polypeptide or a characteristic or biologically active portion of an HA or NA polypeptide. In some embodiments, the invention provides nucleic acids which are complementary to nucleic acids which encode an HA or NA polypeptide or a characteristic or biologically active portion of an HA polypeptide.

In some embodiments, nucleic acids can be DNA or RNA, and can be single stranded or double-stranded. In some embodiments, nucleic acids in accordance with the invention may include one or more non-natural nucleotides; in some embodiments, nucleic acids in accordance with the invention include only natural nucleotides.

Expression of nucleic acid molecules in accordance with the present invention may be regulated by a second nucleic acid sequence so that the molecule is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the nucleic acid molecules of the invention may be controlled by a promoter and/or enhancer element, which are known in the art.

Nucleic acid constructs of the present invention are inserted into an expression vector or viral vector by methods known to the art, and nucleic acid molecules are operatively linked to an expression control sequence.

An expression vector containing a nucleic acid molecule is transformed into a suitable host cell to allow for production of the protein encoded by the nucleic acid constructs. Exemplary host cells include prokaryotes (e.g., *E. coli*) and eukaryotes (e.g., a Vero, COS, 293 or CHO cell). Host cells transformed with an expression vector are grown under conditions permitting production of an engineered HA polypeptide the present invention followed by recovery of an engineered HA polypeptide.

Engineered HA polypeptides of the present invention may be purified by any technique known in the art. For example, not wishing to be bound by theory, engineered HA polypeptides may be recovered from cells either as soluble polypeptides or as inclusion bodies, from which they may be

extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify engineered HA polypeptides of the present invention, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used. Engineered HA polypeptides of the present invention may also be recovered from conditioned media following secretion from eukaryotic or prokaryotic cells.

Conformation of Engineered HA or NA Polypeptides

Engineered HA or NA polypeptides generated according to various methods described herein may be assessed for desired expression and conformation. Screening methods are known to the art and include cell-free, cell-based, and animal assays. In vitro assays can be either solid state or soluble target molecule detection may be achieved in a number of ways known to the art, including the use of a label or detectable group capable of identifying an engineered HA polypeptide which is bound to a target molecule (e.g., immunoglobulin). Detectable labels may be used in conjunction with assays using engineered HA or NA polypeptides of the present invention. For example, engineered HA or NA polypeptide as described herein may be evaluated and selected based on expression and conformational characteristics as determined by assays described in International Patent Application PCT/US2015/033205 entitled "Expression and Conformational Analysis of Engineered Influenza Hemagglutinin" filed on May 29, 2015.

Other binding assays may also be used to evaluate expression and conformation of engineered HA or NA polypeptides, including but not limited to, a Protein Expression and Purification Platform (PEPP) system, or a Biolayer Interferometry (BLI) system. In some embodiments, expression and conformation of engineered HA or NA polypeptides may be measured and ranked by quantitating the level of monoclonal antibody binding.

The present invention also provides methods for testing engineered HA or NA polypeptides in accordance with the invention in an animal host. As used herein, an "animal host" includes any animal model suitable for influenza research. For example, animal hosts suitable for the invention can be any mammalian hosts, including primates, ferrets, cats, dogs, cows, horses, rodents such as, mice, hamsters, rabbits, and rats. In some embodiments, an animal host used for the invention is a ferret. In particular, in some embodiments, an animal host is naïve to viral exposure or infection prior to administration of a binding agent in accordance with the invention (optionally in a composition in accordance with the invention). In some embodiments, the animal host is inoculated with, infected with, or otherwise exposed to virus prior to or concurrent with administration of an engineered HA or NA polypeptide in accordance with the invention. An animal host used in the practice of the present invention can be inoculated with, infected with, or otherwise exposed to virus by any method known in the art. In some embodiments, an animal host may be inoculated with, infected with, or exposed to virus intranasally.

Naïve and/or inoculated animals may be used for any of a variety of studies. For example, such animal models may be used for hemagglutinin inhibition assays, microneutralization assays, challenge assays and virus transmission studies, as is known in the art. It is contemplated that the use of ferrets in virus transmission studies may serve as a reliable predictor for virus transmission in humans. For example, air transmission of viral influenza from inoculated animals (e.g., ferrets) to naïve animals is known in the art (Tumpey et al., 2007, *Science* 315; 655-59; incorporated herein by reference). Virus transmission studies may be used

to test engineered HA polypeptides in accordance with the invention. For example, engineered HA polypeptides in accordance with the invention may be administered to a suitable animal host in order to determine the efficacy of said engineered HA polypeptide in eliciting a broad immune response in the animal host. Using information gathered from studies in an animal host, one may predict the efficacy of an engineered HA polypeptide to elicit broadly protective in a human host.

Pharmaceutical Compositions

In some embodiments, the present invention provides for pharmaceutical compositions including an engineered HA or NA polypeptide as described herein and/or related entities. For example, in some embodiments, engineered HA or NA polypeptides, nucleic acids encoding such polypeptides, characteristic or biologically active fragments of such polypeptides or nucleic acids, antibodies that bind to and/or compete with such polypeptides or fragments, small molecules that interact with or compete with such polypeptides or with glycans that bind to them, etc. are included in pharmaceutical compositions in accordance with the invention.

Such pharmaceutical compositions are typically formulated as immunogenic or vaccine compositions. In some embodiments, the immunogenic compositions are pharmaceutical compositions comprising one or more of the following: (1) inactivated virus, (2) live attenuated influenza virus, for example, replication-defective virus, (3) virus-like particles (VLPs), (4) engineered HA or NA polypeptide, (5) nucleic acid encoding an engineered HA or NA polypeptide or characteristic or biologically active portion thereof, (6) DNA vector that encodes an engineered HA polypeptide in accordance with the invention or characteristic or biologically active portion thereof, and/or (7) expression system, for example, cells expressing one or more influenza proteins to be used as antigens.

In some embodiments, the present invention provides methods of preventing or treating influenza infections by administration of such pharmaceutical compositions in accordance with the invention. In some embodiments, pharmaceutical compositions in accordance with the invention are administered to a subject suffering from or susceptible to an influenza infection. In some embodiments, a subject is an animal, including but not limited to birds (e.g., chickens, ducks, turkeys, etc.), dogs, horses and pigs. In some embodiments, a subject is considered to be suffering from an influenza infection in the subject is displaying one or more symptoms commonly associated with influenza infection. In some embodiments, the subject is known or believed to have been exposed to the influenza virus. In some embodiments, a subject is considered to be susceptible to an influenza infection if the subject is known or believed to have been exposed to the influenza virus. In some embodiments, a subject is known or believed to have been exposed to the influenza virus if the subject has been in contact with other individuals known or suspected to have been infected with the influenza virus and/or if the subject is or has been present in a location in which influenza infection is known or thought to be prevalent.

In some embodiments, subjects suffering from or susceptible to influenza infection are tested for antibodies to engineered HA or NA polypeptides in accordance with the invention prior to, during, or after administration of pharmaceutical compositions in accordance with the invention. In some embodiments, subjects having such antibodies are not administered pharmaceutical compositions comprising engineered HA or NA polypeptides in accordance with the

invention. In some embodiments, an appropriate dose of pharmaceutical composition and/or engineered HA or NA polypeptide is selected based on detection (or lack thereof) of such antibodies.

In some embodiments, selection of a particular subject for treatment, particular engineered HA or NA polypeptide or composition for administration, and/or particular dose or regimen for administration, is memorialized, for example in a written, printed, or electronic storage form.

Compositions comprising an engineered HA or NA polypeptide as described may be administered prior to or after development of one or more symptoms of influenza infection. In some embodiments, a vaccine comprising an engineered HA or NA polypeptide as described herein may be administered prior to or after development of one or more symptoms of influenza infection.

In some embodiments, the present invention provides for treatment of influenza infections by administration of engineered HA or NA polypeptides described herein. In some embodiments, treatment of influenza infections according to the present invention is accomplished by administration of an influenza VLP comprising an engineered HA or NA polypeptide as described herein. In some embodiments, treatment of influenza infections according to the present invention is accomplished by administration of a vaccine. To date, although significant accomplishments have been made in the development of influenza vaccines, there is room for further improvement. The present invention provides vaccines comprising engineered HA polypeptides in accordance with the invention, and particularly comprising engineered HA polypeptides that elicit broadly protective immune responses to multiple neutralizing antigenic determinants (e.g., epitope) of the engineered HA polypeptides.

In some embodiments, the present invention provides for immunogenic compositions (e.g., vaccines) and the administration of these immunogenic compositions to a human subject. In particular embodiments, a human subject is 6 months of age or older, is 6 months through 35 months of age, is 36 months through 8 years of age, or 9 years of age or older.

Thus, in some embodiments, the present invention provides inactivated flu vaccines. In some embodiments, inactivated flu vaccines comprise one of three types of antigen preparation: inactivated whole virus, sub-virions where purified virus particles are disrupted with detergents or other reagents to solubilize the lipid envelope ("split" vaccine) or purified HA polypeptide ("subunit" vaccine). In some embodiments, virus can be inactivated by treatment with formaldehyde, beta-propiolactone, ether, ether with detergent (such as TWEEN-80®), cetyl trimethyl ammonium bromide (CTAB) and Triton N101, sodium deoxycholate and tri(n-butyl) phosphate. Inactivation can occur after or prior to clarification of allantoic fluid (from virus produced in eggs); the virions are isolated and purified by centrifugation (Nicholson et al., eds., 1998, Textbook of Influenza, Blackwell Science, Malden, MA; incorporated herein by reference). To assess the potency of the vaccine, the single radial immunodiffusion (SRID) test can be used (Schild et al., 1975, Bull. World Health Organ., 52:43-50 & 223-31; Mostow et al., 1975, J. Clin. Microbiol., 2:531; both of which are incorporated herein by reference).

In some embodiments, engineered HA polypeptides of the present invention are used as a component of seasonal influenza vaccines or as part of an influenza vaccination regimen intended to confer long-lasting (multi-season) protection. The nucleic acid sequences encoding the engineered influenza HA polypeptides obtained by the methods

described herein can be combined with one or more donor viruses and used in a reverse genetics system to produce an infectious reassortant influenza virus. Reverse genetics systems can be used produce infectious, reassortant viruses, or attenuated viruses from their cDNAs. The reverse genetics methods are well-known by the one skilled in the art and include, but are not limited to, the methods using the plasmids described in Neuman et al, 1999, *Proc Natl Acad Sci USA*, 96(16):9345-9350; Neumann et al, 2005, *Proc Natl Acad Sci USA*, 102(46):16825-16829; Zhang et al, 2009, *J Virol*, 83(18):9296-9303; Massin et al, 2005, *J Virol*, 79(21):1381 1-13816; Murakami et al, 2008, 82(3):1605-1609; and/or the cells described in Neuman et al, 1999, *Proc Natl Acad Sci USA*, 96(16):9345-9350; Neumann et al, 2005, *Proc Natl Acad Sci USA*, 102(46): 16825-16829; Zhang et al, 2009, *J Virol*, 83(18):9296-9303; Massin et al, 2005, *J Virol*, 79(21) :1381 1-13816; Murakami et al, 2008, 82(3):1605-1609; Koudstaal et al, 2009, *Vaccine*, 27(19): 2588-2593; Schickli et al, 2001, *Philos Trans R Soc Lond Biol Sci*, 356(1416):1965-1973; Nicolson et al, 2005, *Vaccine*, 23(22):2943-2952; Legastelois et al, 2007, *Influenza Other Respi Viruses*, 1 (3):95-104; Whiteley et al, 2007, *Influenza Other Respi Viruses*, 1 (4): 157-166.

In certain embodiments, the reverse genetics method may be:

- (i) the 16 plasmid method, such as the method described by Neuman et al, 1999, *Proc Natl Acad Sci USA*, 96(16):9345-9350, and in US 2009/0246830 or US 2011/0143424 (each of which is hereby incorporated by reference in its entirety), in which the influenza virus is produced by transfecting cells, using a polyamine derivative (Trans IT-LT1), with 8 plasmids each containing a cDNA complementary to one influenza vRNA under the control of an RNA polymerase I promoter and an RNA polymerase I terminator, and 8 plasmids each containing a cDNA complementary to one of the PA, PB1, PB2, NP, HA, NA, M and NS mRNAs under the control of RNA polymerase II promoter. In particular, the cells are human kidney embryonic adherent cells (293T cell line);
- (ii) the 12 plasmid method, such as the method described by Fodor et al, 1999, *J Virol*, 73(1 1):9679-9682, and in US 2004/0142003, US 2012/0058538 (each of which is hereby incorporated by reference in its entirety) in which the influenza virus is produced by transfecting a first cell type with 8 plasmids each containing a cDNA complementary to one influenza vRNA under the control of an RNA polymerase I promoter and an RNA polymerase I terminator (hepatitis delta ribozyme), and 4 plasmids each containing a cDNA complementary to one of the NP, PA, PB1 and PB2 mRNAs under the control of RNA polymerase II promoter, and by further amplifying the virus on a second cell type. In particular, said first cell type is Vero cells and said second cell type is MDBK;
- (iii) the 13 plasmid method, such as the method described by De Wit et al, 2007, *Journal of General Virology*, 88:1281-1287 (which is hereby incorporated by reference in its entirety) in which the influenza virus is produced by transfecting cells with 8 plasmids each containing a cDNA complementary to one influenza vRNA under the control of an T7 RNA polymerase promoter and an T7 RNA polymerase terminator, 4 plasmids each containing a cDNA complementary to one of the NP, PA, PB1 and PB2 mRNAs under the control of RNA polymerase II, and one plasmid containing the cDNA complementary to the mRNA encod-

ing the T7 RNA polymerase and a nuclear localization signal under the control of RNA polymerase II. In particular, the transfected cells are Vero, 293T, or QT6 (fibrosarcoma cell line from Japanese quail) cells.

- (iv) the 8 plasmid method, such as the method described by Hoffmann et al, 2000, *PNAS*, 97(1 1):6108-61 13 and in WO 01/83794 (each of which is hereby incorporated by reference in its entirety) in which each plasmid is capable of expressing both mRNA and vRNA(s). Thus each plasmid contains cDNA complementary to one influenza vRNA and two transcription cassettes instead of one as in the preceding case. The cDNA complementary of each of the eight influenza virus vRNAs is inserted between the polymerase I terminator and the polymerase I promoter. This polymerase I transcription unit is flanked by the polymerase II promoter and a polyadenylation signal. The first transcription cassette allows the transcription of cDNA in the form of a vRNA. The second transcription cassette allows the transcription of cDNA in the form of mRNA which is then translated into viral protein(s) using the cellular machinery. With the aid of this double cassette system for transcription, also called Pol I-Pol II system, the cDNA of the same plasmid is transcribed both in the form of vRNA and in the form of mRNA. This manifests itself at the level of the transfected cell by the expression of a vRNA and of one or more viral proteins. In particular, a co-culture of adherent MDCK cells and of 293T cells and, as transfection agent, a polyamine derivative (Trans IT-LT1) are used.
- (v) the 3 plasmid method, such as the method described by Neumann et al, 2005, *PNAS*, 102(46): 16825-16829 (which is hereby incorporated by reference in its entirety), in which the influenza virus is produced by transfecting cells with one plasmid containing the 8 cDNAs complementary to PB2, PB1, PA, HA, NP, NA, M and NS vRNAs each under the control of an RNA polymerase I promoter and a polymerase I terminator and 2 plasmids, the first one containing the 3 cDNA complementary to one of the PB2, PB1 and PA mRNAs and the second one containing the cDNA complementary to the NP mRNA, under the control of a RNA polymerase II promoter. In particular, the transfected cells are 293T or Vero.
- (vi) the 1 plasmid method, such as the method described by Zhang et al, *J. Virol.*, 83(18): 9296-9303 (which is hereby incorporated by reference in its entirety), in which the influenza virus is produced by transfecting cells with one plasmid containing the 8 cDNAs complementary to PB2, PB1, PA, HA, NP, NA, M and NS vRNA under the control of murine polymerase I terminator and a chicken RNA polymerase I promoter and with a polymerase II promoter and a polyadenylation signal between PB2, PB1, PA and NP cDNAs. In particular, the transfected cells are CEF cells.
- (vii) the method described in WO 2005/062820 (which is hereby incorporated by reference in its entirety) using two different cellular systems: in a first step, cells are transfected with 8 bidirectional plasmids with the Poll-Poll system (Pol/Poll) and then in a second step, the transfected cells are cultured with cells from another cell line that is very permissive for the influenza virus in order to amplify the production of the influenza virus. In particular, said transfected cells in the first step are Vero cells, and said other cell line in the second step are CEK or CEF cell lines which are lines derived from chicken embryo cells.

In some embodiments, prior to being used in the reverse genetics methods described above, nucleic acid sequences encoding the engineered influenza HA polypeptides may be further optimized according to the method described in U.S. provisional application 62/172,949, incorporated by reference herein.

In some embodiments, influenza virus for use in vaccines is grown in eggs, for example, in embryonated hen eggs, in which case the harvested material is allantoic fluid. Alternatively or additionally, influenza virus or engineered hemagglutinin polypeptides may be produced from any method using tissue culture to grow the virus. Suitable cell substrates for growing the virus or otherwise recombinantly producing the engineered hemagglutinin polypeptides include, for example, dog kidney cells such as MDCK or cells from a clone of MDCK, MDCK-like cells, monkey kidney cells such as AGMK cells including Vero cells, cultured epithelial cells as continuous cell lines, 293T cells, BK-21 cells, CV-1 cells, or any other mammalian cell type suitable for the production of influenza virus (including upper airway epithelial cells) for vaccine purposes, readily available from commercial sources (e.g., ATCC, Rockville, Md.). Suitable cell substrates also include human cells such as MRC-5 cells. Suitable cell substrates are not limited to cell lines; for example primary cells such as chicken embryo fibroblasts are also included.

Engineered hemagglutinin polypeptides may also be expressed/produced in diverse eukaryotic-based expression systems, including microalgae (e.g. *Schizochytrium* sp.; see, e.g., Bayne, A-C.V. et al., PLOS ONE, 8(4):e61790, April 2013), plant-based systems (e.g., tobacco plants; see, e.g., Jul-Larsen, A., et al., Hum Vaccin Immunother., 8(5):653-61, 2012), yeast (see, e.g., Athmaram, T. N. et al., Virol J., 8:524, 2011), and fungi (see, e.g., Allgaier, S. et al., Biologicals, 37:128-32, 2009). Bacterial based expression systems are also encompassed by the present invention (see, e.g., Davis, A. R. et al., Gene, 21:273-284, 1983).

In some embodiments, vaccines in accordance with the invention further comprise one or more adjuvants. For example, aluminum salts (Baylor et al., 2002, Vaccine, 20:S18; incorporated herein by reference) and monophosphoryl lipid A (MPL; Ribic et al., 1986, Immunology and Immunopharmacology of Bacterial Endotoxins, Plenum Publ. Corp., NY, p. 407; incorporated herein by reference) can be used as adjuvants in human vaccines. Alternatively or additionally, new compounds are currently being tested as adjuvants in human vaccines, such as AS03, MF59, and saponins such as QS21.

Additionally, some adjuvants are known in the art to enhance the immunogenicity of influenza vaccines, such as poly[di(carboxylatophenoxy)phosphazene] (PCCP; Payne et al., 1998, Vaccine, 16:92; incorporated herein by reference), interferon- γ (Cao et al., 1992, Vaccine, 10:238; incorporated herein by reference), block copolymer P1205 (CRL1005; Katz et al., 2000, Vaccine, 18:2177; incorporated herein by reference), interleukin-2 (IL-2; Mbwuikie et al., 1990, Vaccine, 8:347; incorporated herein by reference), and polymethyl methacrylate (PMMA; Kreuter et al., 1981, J. Pharm. Sci., 70:367; incorporated herein by reference).

In addition to immunogenic compositions (e.g., vaccines comprising one or more of the engineered influenza hemagglutinin polypeptides described herein), the present invention provides other therapeutic compositions useful in the treatment of viral infections. Therapeutic compositions include, for example, influenza VLPs, fusions proteins, and an engineered HA polypeptide itself as described herein.

In some embodiments, any of the immunogenic compositions (e.g., vaccines) described herein offer broad cross-protection against different varieties of influenza viruses. For example, in some embodiments, immunogenic compositions described herein offer cross-protection against pinipeds and/or human-adapted influenza B viruses. In some embodiments, any of the immunogenic compositions described herein offer cross-protection against one or more influenza B lineage strains. In some embodiments, the immunogenic compositions described herein provide cross-protection against multiple strains of influenza B lineages (see, e.g., FIG. 3).

Pharmaceutical compositions of the present invention can be administered by a variety of routes, including oral, intravenous, intramuscular, intra-arterial, subcutaneous, intraventricular, transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, or drops), mucosal, nasal, buccal, enteral, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. In general the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (e.g., its stability in the environment of the gastrointestinal tract), the condition of the patient (e.g., whether the patient is able to tolerate oral administration), etc.

In some embodiments parenteral administration, such as subcutaneous, intravenous or intramuscular administration, is achieved by injection. In some embodiments, injectables are prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. In some embodiments, injection solutions and suspensions are prepared from sterile powders, granules, and.

General considerations in the formulation and manufacture of pharmaceutical agents may be found, for example, in *Remington's Pharmaceutical Sciences*, 19th ed., Mack Publishing Co., Easton, PA, 1995; incorporated herein by reference.

Pharmaceutical compositions in accordance with the invention may be administered in any dose appropriate to achieve a desired outcome. In some embodiments, the desired outcome is induction of a lasting adaptive immune response against multiple influenza strains. In some embodiments, the desired outcome is reduction in intensity, severity, and/or frequency, and/or delay of onset of one or more symptoms of influenza infection.

In some embodiments, pharmaceutical compositions in accordance with the invention are administered in single or multiple doses. In some embodiments, pharmaceutical compositions in accordance with the invention are administered in multiple doses administered on different days (e.g., prime-boost vaccination strategies). In some embodiments, pharmaceutical compositions in accordance with the invention are administered according to a continuous dosing regimen, such that the subject does not undergo periods of less than therapeutic dosing interposed between periods of therapeutic dosing. In some embodiments, pharmaceutical compositions in accordance with the invention are administered according to an intermittent dosing regimen, such that the subject undergoes at least one period of less than therapeutic dosing interposed between two periods of therapeutic dosing.

In some embodiments, a dose administered to a subject should be sufficient to induce a beneficial therapeutic response in a subject over time, or to inhibit or prevent influenza B virus infection. In some embodiments, the dose

comprises 15 µg of one of the engineered influenza B HA polypeptides described herein. In some embodiments, the dose comprises 7.5 µg of one of the engineered influenza B HA polypeptides described herein. In some embodiments, the dose comprises 3.0 µg of one of the engineered influenza B HA polypeptides described herein. The dose required may vary from subject to subject depending on the species, age, weight and general condition of the subject, the severity of the infection being treated, the particular composition being used and its mode of administration.

The present invention will be more fully understood by reference to the following Examples. All literature citations are incorporated by reference.

EXAMPLES

These Examples are set forth to aid in the understanding of the invention but are not intended to, and should not be construed to limit its scope in any way. The Examples do not include detailed descriptions of conventional methods that would be well known to those of ordinary skill in the art (molecular cloning techniques, etc.). Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1. Design and Methodology for Construction of Hemagglutinin (HA) Polypeptides

The present Example describes the design and production of engineered HA polypeptides that elicit broad neutralizing immune responses. The engineered HA polypeptides combine multiple B-cell epitopes from different hemagglutinin sequences to create mosaic antigens. The present Example specifically illustrates construction of engineered HA polypeptides that combine HA sequences from influenza B lineages and strains. These mosaic antigens are predicted to confer cross-protection against multiple strains by maximizing sequence homology for at least one neutralizing epitope. Further, these mosaic antigens can be used alone or in combination with other influenza antigens, as a component of seasonal influenza vaccines, or as part of a vaccination regimen intended to confer long-lasting, multi-season protection against influenza infection.

Additional Examples presented herein demonstrate the successful immune response elicited by these engineered HA polypeptides, as assayed by HA inhibition using sera from immunized animals. These Examples demonstrate the potential of the presently described methodology for the design and production of engineered HA polypeptides and provide the next-generation of broadly cross-reactive and neutralizing influenza vaccines.

FIG. 6 sets forth an exemplary flowchart of the methodology for the design and production of engineered HA polypeptides as described herein. Principal Component Analysis to Assess Cross Reactivity of Influenza B HAs

Principal Components Analysis (PCA) is a common technique for working with high dimensional data and highlighting patterns in the data (i.e. it can be used to simplify large datasets and facilitate data exploration and visualization). Applied to biological sequences (proteins, genes), the technique enables comparison of thousands of sequences and the identification of groups of similar sequences based on a measure of sequence dissimilarity (Hamming distance, percent identity, percent similarity, surface accessibility, etc.). In the case of Human influenza B viruses, hemagglutinin

(HA) protein sequences were obtained from the NCBI Influenza Virus Resource database, trimmed to remove signal peptides, transmembrane regions and cytoplasmic tails and the resulting ectodomain sequences were aligned.

The pair-wise dissimilarity matrix was calculated from the multiple sequence alignment based on the Hamming distance between pairs of sequences with no prior assumptions regarding function or structure of the sequences. Principal Components Analysis (PCA) was applied to the dissimilarity matrix for the purpose of dimension reduction and to facilitate visualization of the relative distances between HA proteins. The first 2-3 principal components were retained for visualizing protein relationships in sequence space and represent a reasonable approximation of the general structure of the phylogenetic tree. Calculations were performed using custom scripts written in python and R.

Visualization of the first 2 principal components identifies clear patterns associated with influenza B hemagglutinins including the two influenza B lineages (Yamagata vs Victoria). Additionally, sequences form distinct clusters based on similarity. Reference and vaccine strains are highlighted in FIG. 5 as are engineered SMART HA sequences (grey diamonds) which show little overlap with the clusters consisting of circulating strain HA sequences.

A broadly protective antigen should be cross-reactive across multiple clusters within and between lineages (FIG. 5). Modifications designed into the engineered HAs were deduced from an in silico analysis of sequence variation in both past and current circulating influenza strains. This analysis included mapping antigenic and epitope patterns as well as structural modeling of the HA protein. Targeted changes were subsequently introduced at precise amino acid residue locations and/or specific regions of the protein with known immune profiles in order to yield novel influenza B HA polypeptides that would be reactive across the sequence clusters illustrated in FIG. 5.

Each novel mosaic design was composed of multiple neutralizing hemagglutinin B-cell epitope patterns derived from antigenically diverse influenza B strains (including both Yamagata and Victoria lineages). The mosaic pattern of B-cell epitopes were assembled onto a backbone hemagglutinin sequence. One of the following nine backbone hemagglutinin sequences was used: B/Hong Kong/330/2001, B/Hong Kong/05/1972, B/Lee/40, B/Massachusetts/02/2012, B/Panama/45/1990, B/Singapore/222/79, B/Victoria/02/1987, B/Yamagata/16/1988, B/Brisbane/60/2008. The selected backbone provides the inter-epitope sequence of the engineered construct as well as the signal peptide and transmembrane domains required for full-length hemagglutinin molecules that are expressed and functional.

Structural Mapping of Antigenic Repertoires

The approach used here to construct mosaic hemagglutinin sequences is termed 'SMART' for Structural Mapping of Antigenic Repertoires (an overview of the process is presented in FIGS. 7 and 8). Briefly, known neutralizing and non-neutralizing hemagglutinin B-cell epitopes described for any B virus were mapped to corresponding regions of Influenza B HA. Individual sequences for each B-cell epitope were extracted and enumerated from the sequences of all available circulating Influenza B strains to generate an 'antigenic repertoire'. Four distinct SMART workflows were developed to combine antigenic repertoires into novel mosaic hemagglutinin molecules that are distinct from natural circulating strains. An overview of the compiled SMART workflow is presented in FIG. 8.

FIG. 9 provides a demonstration of the complete process to generate a SMART Dominant HA design. Table 5 provides

epitope sequence patterns represented in FIG. 9. (SEQ ID NOS 46-59 and 59-62, respectively, in order of appearance).

TABLE 5

Epitope Sequence Patterns	Counts
GKAPLKPESLTSDGDPVH	8898
GKAPLNPELLKNEGNPMD	1007
GKAPLNPELLTNEGNPMD	880
GKAPLKPETLTSDGDPVH	221
GKAPLNPELLTNEGNPMG	145
YDNKGVTAkWVKKGNSHSTSADQSLQIDQE	6651
YDNKGVTAkWVKKGNSHSTTADQSLQISQE	1423
YTT-GVSASWTGKNGLHFNIGDRALHKDQE	602
YDNKGVTAkWVKKGNSHSTSADQSLQIEQE	382
YTT-GVSASWTGKNGLHFNIGDKALHKDQE	307
SHNGESRD	9012
SHNGKSRD	981
PHAGAKRE	806
PHAGAKRG	323
PHAGAKRG	155
YHHS�VDGWLTQΑIDITKVNVT	9799
YHHS�VDGWQTQAINITKVNVT	1903
YHHS�IDGWQTQAINITKVNVT	216

Structural Modeling and Selection of Designs

One aspect of the SMART workflow for the design of mosaic antigens is structure-based molecular modeling to identify designs with conformational stability, which are then selected for experimental validation. A summary of the modeling and selection process is provided in FIG. 10. Three-dimensional coordinates of a high-quality structure of influenza B hemagglutinin (HA) were used as the template for modeling the structural backbones into which epitope repertoires were inserted (PDB ID: 4M40).

The insertion of epitopes into the 3D structure of the backbone molecule was performed using the design protocol of the Rosetta molecular modeling software version 3.1 (Simons et al. J. Mol. Biol. 1997 268:209-225; Leaver-Fay et al. Methods Enzymol. 2011 487:545-574). Following epitope insertion, the total energies of the resulting designed molecules were calculated using the Rosetta energy function shown in FIG. 11. Molecules with negative total energy scores were predicted to have a good probability of folding into stable proteins while those with positive energy scores were considered less likely to fold properly. FIG. 12 shows a scatter plot of energy score vs the model number of computationally predicted SMART structural models. For clarity, only the top scoring 2,500 out of a total of 17,500 modeled structures are displayed on the plot.

Of the 17,500 structural models generated, one hundred lowest energy models were shortlisted for experimental testing. Fifty of the short-listed candidates were prioritized for experimental validation to assess stable soluble expression, proper folding and immunogenicity (Table 6). Thus, the approaches described above yielded novel hemaggluti-

nin molecules for the Influenza B lineage strains that do not match naturally occurring strains. These novel sequences are designed to provide broader coverage to naturally occurring strains than existing vaccine strains (including being cross-protective across the two antigenically distinct lineages). The resulting vaccine candidates can be further modified by targeted engineering of the sequence (including engineering glycosylation patterns, modifying stability or modifying specific epitopes).

TABLE 6

B/Bris/60/08
br08_CH
br08_CO1
br08_DO1
br08_DO2
br08_DO3
br08_RA45
br08_RA51
br08_RA65
br08_RA8
B/HK/05/72
hk72_CH
hk72_DO3
B/Mass/02/12
ma12_CH
ma12_CO1
ma12_DO2
ma12_DO3
ma12_RA102
ma12_RA26
ma12_RA33
ma12_RA40
ma12_RA46
ma12_RA65
ma12_RA67
ma12_RA81
ma12_RA82
ma12_RA97
B/HK/330/01
hk01_RA83
B/Pan/45/90
pan90_DO2
pan90_RA101
pan90_RA17
pan90_RA20
pan90_RA3
pan90_RA35
pan90_RA82
pan90_RA83
B/Sing/222/79
sing79_CO1
sing79_DO1
sing79_RA101
sing79_RA103
sing79_RA26
sing79_RA34
sing79_RA41
sing79_RA57
sing79_RA68
sing79_RA74
sing79_RA75
sing79_RA76
sing79_RA80

TABLE 6-continued

B/Yam/16/88
yam88_DO1
yam88_RA25
yam88_RA39
yam88_RA68
yam88_RA86
B/Vic/87
vic87_DO2

SMART Design Guided Engineered Soluble Recombinant HA (rHA)

Fifty soluble versions of the Influenza B SMART HA proteins were synthesized, expressed and purified from HEK293 cells using the protein expression and purification platform (PEPP). SMART HA designs were engineered as full-length trimeric HA proteins. Designs were modified for expression of soluble recombinant protein by replacement of the transmembrane region and cytoplasmic domain with thrombin cleavage site, foldon trimerization domain and his-tag. (FIG. 13). Approximately 50% of the novel designs were purified as soluble versions (FIG. 14).

In Vitro Recognition of PEPP Influenza B rHAs

Purified, soluble Influenza B SMART HAs were evaluated for their ability to bind Influenza B-specific monoclonal antibodies. Using the ForteBio Octet system, verification of binding to conformational epitopes is performed at a single concentration. Several of these engineered mosaic antigens are able to bind Influenza B specific monoclonal antibodies indicating that the molecules are well-folded (and likely functional). A representative data set demonstrating positive binding of wild type and engineered rHAs to an influenza B monoclonal antibody is shown in FIG. 15.

Example 2. Structural Mapping of Antigenic Repertoires (SMART) Workflow

One rational design approach to creating a broadly protective HA-based vaccine is to include epitopes from as many viral isolates as possible in a polyvalent vaccine. The methodology behind the generation of mosaic B-cell epitope sequences is termed SMART for the 'Structural Mapping of Antigenic Repertoires'. The first stage of SMART relies on the identification and classification of known B-cell epitopes for the influenza A hemagglutinin molecule. The epitopes and antigenic sites for Influenza A hemagglutinin were subdivided into three classes (tiers): 1) epitopes supported by 3D-structural mapping of the contact sites from neutralizing antibodies; 2) neutralizing epitopes not supported by 3D mapping of contact sites; and 3) classical antigenic regions that lack precise mapping of epitope residues. A polyvalent vaccine with M proteins supports the inclusion of M epitope sequence patterns for each epitope site. Each epitope site with N unique epitope sequences has multiple possible combinations of subsets of M unique epitope sequence patterns:

$$C_M^N = \frac{N!}{M!(N-M)!}$$

This was reduced to M subsets, or cassettes, per epitope site by creating a cassette for each unique epitope sequence pattern and optimizing the selected epitope sequence patterns to optimize alignment coverage. There were M pos-

sible cassettes for R epitopes sites, creating R^M possible combinations. Mosaic sequence templates were generated for large numbers of random combinations of cassettes to sample a subset of the R^M possible combinations. The alignment coverage for nine geographic regions, viral isolate years, and sub-family clusters was evaluated for each mosaic sequence template. The set of mosaic sequence templates were then combined with target backbone sequence(s) and subjected to structural refinement to generate candidate mosaic polyvalent sequences for vaccine development.

A low fidelity polymerase enables viruses to evolve over multiple generations to evade immunological memory of hosts. This creates a diverse population of related viruses. Vaccines developed to target specific viral isolates may not protect against infection from different isolates of the same virus. The SMART approach for developing broadly protective antigens creates polyvalent mosaic sequences that include B-cell epitopes from as many viral isolates as possible. Starting with known neutralizing antibody epitopes and antigenic sites, SMART identifies corresponding antigenic repertoires ($A_{1-N}, B_{1-N}, C_{1-N}, \dots$) from an alignment of N sequences and generates combinations ($A_{23}, B_{12}, C_{55}, \dots; A_{15}, B_{38}, C_{27}, \dots$) from the repertoires to create sets of M mosaic sequences. To optimize the epitope sequence patterns selected, an ordered subset of M epitope sequence patterns were generated for each unique epitope sequence pattern with order optimized for alignment coverage. For each unique epitope sequence pattern, E_j , additional epitope sequence patterns ($E_k, E_m, \dots$) from the same epitope site were selected to maximize the alignment coverage using a scoring matrix (e.g., blosum80.mat). Ordered epitope sequence patterns cassettes from different epitope sites were randomly combined to create large numbers of mosaic template sequences (e.g., for classical antigenic sites, Ca, Cb, Sa, and Sb [Igarashi, et al., 2009]: $Ca_{1_i}, Ca_{2_j}, Cb_{k_i}, Sa_{l_j}, Sb_{m_i}, \dots$ represents the selected cassettes for one mosaic template set of sequences).

To avoid over-representation biases in the available sequences, principal component analysis (PCA) was used to define virus sub-family clusters. The best mosaic sequence templates were selected by evaluating overall alignment coverage by geographic regions, viral isolate years, and PCA clusters. The selected set of mosaic template sequences were combined with target backbone sequences to generate the set of full-length mosaic protein sequences. Structure refinement of these mosaic sequences yields the final set of vaccination proteins. An overview of the mosaic approach is shown in FIG. 16.

Create Multiple Sequence Alignment

All available full-length hemagglutinin protein sequences were downloaded from the NCBI's Influenza Virus Resource (<http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html>) (Bao et al., 2008). Full-length sequences were sub-divided for host and virus sub-type. For the construction of mosaic antigens a host range restriction to human was applied to each dataset. In the case of the human H1N1 viral sub-type sequences, x number of sequences were available as of Apr. 13, 2011. Redundant sequences were removed using CDHIT (Fu et al., 2012, CD-HIT: accelerated for clustering the next-generation sequence data, Bioinformatics, 28 (23):3150-3152; Weizhong and Dodzik, 2006, Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences, Bioinformatics, 22(13): 1658-1659; Weizhong et al., 2002, Tolerating some redundancy significantly speeds up clustering of large protein databases, Bioinformatics, 18(1):77-82; Weizhong et al., 2001, Clustering of highly homologous sequences to reduce

the size of large protein databases, *Bioinformatics*, 17(3): 282-283) and sequences containing ambiguous residues (X, J, B, Z) were excluded to yield a final, non-redundant set of 2043 H1N1 hemagglutinin sequences. The non-redundant sequences were initially aligned MAFFT (e.g., see Katoh and Standley, 2013, *Mol. Biol. Evol.* 30(4):772-780, or Katoh et al., 2002, *Nucleic Acids Res.* 30:3059-3066) and then manually reviewed and edited as required. The manually edited multiple sequence alignment was used for the identification of sequence clusters and for the construction of mosaic sequences.

Identify Epitopes for Target Protein of Interest

Target human B-cell antibody epitopes were identified from crystal structures of hemagglutinin with neutralizing antibodies (Table 2), the Immune Epitope Database (IEDB; Yang, et al., 2009), and antigenic sites from literature (Igarashi et al., 2010). Linear and discontinuous epitopes were organized into a text file format based on the IEDB epitope export Excel file format. Identified epitopes were classified on the basis of supporting publications and structural models into one of three tiers. Tier 1 consists of neutralizing epitopes for which 3D structural models of antigen-antibody contact sites were available. Tier 2 of epitopes includes neutralizing epitopes not supported by 3D structural models. And, tier 3 includes the classical antigenic regions which lack precise characterization of epitope regions.

Antigenic regions can have overlapping epitopes (FIGS. 17A and 17B). Epitope sites for 97823, 97824, and 99799 (IEDB numbering) were nearly identical. The residue positions for 97823 were a subset of the positions for 97824. Epitope 97844 has N₃₅, L₃₆, and D₄₆ not covered by 99799 and 9799 has V₃₆₄ not covered by 97824. For overlapping epitope, the order in which the epitopes were layered into the mosaic templates is important. The residues in the mosaic sequences were determined by the order that the epitopes were added to the templates. For two or more overlapping epitopes, residue positions defined by previously added epitopes mask a subset of the positions for the subsequently added overlapping epitopes. One alternative is to allow the ordered layering of overlapping epitope sequence patterns that were derived from different source viral isolates resulting in mosaic sequences with hybrid epitopes not reflected in the viral isolates in the alignment. Alternatively, overlapping epitopes can be combined and selected consistently from the same viral isolates. For example, adding V364 from 99799 with 97824 generates a combined antigenic site for 97823, 97824, and 99799 (FIG. 17A). Similarly, epitopes 137355, 138194, 138269, and 97791 can be combined into one or two combined sites (FIG. 17B). This second approach was used to resolve overlapping epitopes.

Antigenic Repertoires Identification

To identify corresponding sequence patterns in an epitope across multiple strains, a multiple sequence alignment was searched with linear and discontinuous epitopes. When an epitope or antigen site could be identified within the alignment, the corresponding alignment positions were used for all sequences in the alignment to identify all epitope sequence patterns. For linear epitope peptides with no perfect matches in the alignment, limited sequence mismatches (formula: maximum number of mismatches allowed was (linear site length-5)/2) were permitted. The set of unique epitope sequence patterns was identified for each epitope site. An exemplary flow chart of mosaic sequence generation is shown in FIG. 18.

Cassette Subsets of Unique Epitope Sequence Patterns

SMART supports the development of polyvalent vaccines with varying numbers of mosaic constructs. A total of M unique epitope sequence patterns for each epitope site can be included in a polyvalent vaccine of M mosaic proteins. A cassette for each unique epitope pattern can be generated (for example, computationally generated) by adding additional epitope sequence patterns in an alignment coverage optimization order. A cassette was created for each unique epitope sequence with that sequence being the first in the cassette. Additional sequence patterns from the same site were added to each cassette by selecting the next sequence pattern with maximum alignment coverage determined with a scoring matrix (e.g., blosum80). Up to 20 unique sequence patterns were added to each cassette.

Create Mosaic Templates from Cassettes

Sets of mosaic template sequences were generated by randomly combining cassettes (e.g., Ca1_i, Ca2_j, Cb_k, Sa_p, Sb_m, . . .). The ith epitope sequence pattern in each cassette was layered onto the ith mosaic template in each set.

Evaluate Alignment Coverage of Mosaic Epitope Templates

To avoid potential biases, inherent in the sequence datasets, alignment coverage for viral isolation years, viral geographic regions (Table 6), and sub-family clusters were all evaluated. Multiple sequence alignment coverage by each set of mosaic template sequences was characterized by exact matches of epitope sequence patterns within the alignment. To optimize the selection of the best combinations of epitope cassettes, only the first five mosaic template sequences were used in the evaluation of alignment coverage. Also, only the first five tier 1 epitopes were evaluated in the calculation of alignment coverage to keep the coverage calculations below 100% for the mosaic templates. The best mosaic templates were evaluated by selecting the highest coverage mosaics from the coverage summary. The best set of mosaic templates were extracted from the very large file of mosaic template sequences. An exemplary flowchart of the process overview is shown in FIG. 19.

TABLE 6

Geographic Region	Number of Isolates
Africa	27
Asia	136
China	103
Europe	186
Japan	72
Middle East	36
North America	184
Oceania	42
South America	58

Epitope Sequence Pattern Swapping Optimization

To further optimize the best mosaic template sequences set, the epitope sequence patterns within the mosaic templates can be modified and the mosaic templates regenerated. For the epitope sequence patterns in the first of the mosaic sequence templates, nine alternative sequence patterns were substituted for each of the five scored epitopes to evaluate possible alignment coverage improvements.

Combine Mosaic Epitope Template with Target Backbone Templates

The mosaic epitope sequence templates can be combined with desired viral protein backbone sequence(s).

Structure Refinement

The mosaic sequences were checked for structural consistency using structural bioinformatics tools. High-resolution three-dimensional structures of HA molecules were used as the template for generating the structural backbones

into which epitope repertoires were inserted. The insertion of epitopes into the 3D structure of the backbone molecule was performed using the design protocol of the Rosetta molecular modeling software version 3.1 (Simons et al. J. Mol. Biol. 1997 268:209-225; Leaver-Fay et al. Methods Enzymol. 2011 487:545-574). Following epitope insertion, the total energies of the resulting designed molecules were calculated. Molecules with negative total energy scores were predicted to have a good probability of folding into stable proteins while those with positive energy scores were considered less likely to fold properly and were therefore discarded. Sequences of the one hundred lowest energy models were shortlisted for experimental testing.

Results

Principal component analysis (PCA) was used to separate the H1N1 hemagglutinin sequences into eight unevenly distributed sub-family clusters (FIG. 20). Clusters 1, 3, and 4 were well represented and clusters 2, 7, and 8 were sparsely represented (Table 8).

Evaluating all possible combinations of unique epitope sequence patterns for multiple epitope sites is an N-P complete complexity computational problem. Sampling random combinations of cassettes enables the evaluation of a subset of all possible combinations. Millions of random combinations were generated and the combinations with the highest coverage of the alignment were examined. Top combinations identified are illustrated in Table 8.

TABLE 8

Mosaic	Regions	Years	Clusters	Cluster 2	Cluster 7	Cluster 8
HC578810	98%	90%	93%	10/13 (77%)	20/24 (84%)	15/18 (84%)
HB949180	98%	89%	92%	8/13 (61%)	20/24 (84%)	16/18 (89%)
ID316526	98%	89%	92%	8/13 (61%)	20/24 (84%)	16/18 (89%)
JE56022	98%	89%	92%	8/13 (61%)	20/24 (84%)	16/18 (89%)

Conclusions

The SMART approach enables the sampling of the known antigenic repertoires in mosaic sequences with promising potential for generating broadly protective vaccines.

Example 3. In Vivo Efficacy of Engineered Mosaic Influenza a HA and NA Polypeptides

This Example illustrates that engineered HA polypeptide made in accordance with the previous examples elicited immune responses in the form of broad antibody responses against several influenza strains.

Preparation of Virus-Like Particles (VLPs) Containing Engineered Mosaic Hemagglutinins (HAs)

Influenza VLPs were prepared by three-plasmid transient transfection of HEK293T cells in serum-free Freestyle293 medium. Plasmids encoding engineered mosaic Influenza HA polypeptide sequence as well as those for NA, and HIVgag were mixed at 1:1:1 ratio and used to transiently transfect the HEK293T cells. Culture supernatant was harvested 120 hours post-transfection and VLPs in the supernatant were pelleted by ultracentrifugation over a 20% sucrose cushion and resuspended in PBS.

Immunization of Mice with VLPs Expressing Engineered Mosaic HAs

To assess immunogenicity of engineered mosaic HA designs, groups of 6-8 week old female BALB/c mice were immunized with 5 µg of influenza VLPs or vehicle alone (PBS). All immunizations were formulated as emulsions with an oil-in-water adjuvant, and were delivered subcutaneously in a total volume of 100 µl. Each group received an identical booster dose 21 days after the initial immunization.

Pre-immune and post-immune serum was collected from each animal on days 0 and 35, respectively. Serum pools used for analysis were prepared by mixing equal volumes of serum from each animal within a group.

Hemagglutination Inhibition (HAI) Assay

Replicate serial dilutions of pooled serum from each group were mixed with 4 hemagglutination units of the indicated virus and incubated at room temperature for 30 minutes in a round bottom plate. Each serum/virus mixture was then mixed with an equal volume of 0.5% turkey erythrocytes in saline. The plates were scored when control wells lacking serum demonstrated complete hemagglutination (~30 min). The HAI titer was defined as the maximum serum dilution resulting in complete inhibition of hemagglutination in 50% of the wells tested. FIG. 21 sets forth representative serum HAI titer induced against H1N1 influenza strains for engineered mosaic VLPs.

Microneutralization (MN) Assay

Replicate serial dilutions of pooled serum from each group were mixed with 100 50% tissue culture infectious doses (TCID50) of the indicated virus and incubated at 37° C. for one hour. Each serum/virus mixture was then added to confluent monolayers of madin darby canine kidney (MDCK) cells and incubated at 37° C. for 24 hrs. The monolayers were then fixed and infected wells were identified based on ELISA detection of influenza nucleoprotein. The MN titer was defined as the highest dilution of serum resulting in complete neutralization of virus infection in 50% of the wells tested. FIG. 22 sets forth representative serum MN titer induced against H1N1 influenza strains for engineered mosaic VLPs.

Results

Taken together, these data demonstrate that engineered HA polypeptides as described herein promote broad immunity against H1N1 influenza strains. These exemplary HA polypeptides were developed using a novel computational strategy to create mosaic HA antigens based on conserved repertoires, referred to as SMART. Exemplary HA polypeptides SP8 and SP9 (Table 9) were presented in the context of an HIVgag VLP to perform immunogenicity studies in murine animals. Exemplary engineered mosaic HA polypeptides induced antibody responses predicted to provide protection against both A/California/07/2009 (the currently circulating H1N1 strain) as well as a historical swine flu strain, A/New Jersey/10/1976. More importantly, SP9 induced a broad antibody response, with significant titers induced against vaccine strains dating back to 1986. These data suggest that a vaccine utilizing the engineered mosaic design (e.g., SP9) may have been sufficient for protection from H1N1 strains circulating between the years of 1986 and 2007. Further, a combined immunization incorporating both SP8 and SP9 HA designs would potentially be effective against all H1N1 viruses that have been in circulation since 1986. Thus, the engineered mosaic HA designs as described herein provide candidate HA polypeptides for use in a universal H1N1 vaccine that can be used alone or in combination to induce immunity against a broad array of H1N1 viruses.

TABLE 9

Engineered HA Antigenic Region Sequence	
SP1	GVTASSWLTHHPSNGDQQTLKDQEGR (SEQ ID NO: 11)
SP2	GVSASSWLTHHPSTADQQTLKDQEGR (SEQ ID NO: 12)
SP3	GVTASKWLVHHPSTADQQSLKDQEGR (SEQ ID NO: 13)
SP4	GVSASSWLTHHPPNGDQQRALKDQEGR (SEQ ID NO: 14)
SP5	GVSASSWLTHHPPNGDQKTLKDQEGR (SEQ ID NO: 15)
SP7	GVSAASWLTHHPSTADQQTLKDQEGR (SEQ ID NO: 16)
SP8	GVTAASKWLVHHPSTADQQSLKDQEGR (SEQ ID NO: 17)
SP9	GVSAASWLTHHPPNGDQQRALKDQEGR (SEQ ID NO: 18)
SP10	GVSAASWLTHHPPNGDQKTLKDQEGR (SEQ ID NO: 19)
SP1	CYPTVTGVTASCSKSSFLWLTGVHHPSNIGDQQTLYQEIAKRPKVR DQEGRMNI (SEQ ID NO: 20)
SP2	CYPDVTGVSASCSASSFLWLTKVHHPSTIADQQTLYHEIAIRPKVRD QEGRINI (SEQ ID NO: 21)
SP3	CYPDSNGVTASCPAKSFLWLKVHHPSTSADQQSLYQEIAIRPKVR DQEGRMNI (SEQ ID NO: 22)
SP4	CYPTVTGVSASCSKSSFLWLTGVHHPPNIGDQRALYHEIAKRPKVR DQEGRINI (SEQ ID NO: 23)
SP5	CYPTVTGVSASCPRESSFLWLTGVHHPPNIGDQKTLYHEIAKRPKVRD QEGRINI (SEQ ID NO: 24)
SP6	CYPTVTKGVTAACSKSSFIWLTGIIHHPSNIGDQQTLYQEIAKRPKVR DQEGRMNV (SEQ ID NO: 25)
SP7	CYPDVTKGVSAACSASSFIWLTKIIHHPSTIADQQTLYHEIAIRPKVRD QEGRINV (SEQ ID NO: 26)
SP8	CYPDSNKGVTAACPAKSFIWLVKIIHHPSTSADQQSLYQEIAIRPKVR DQEGRMNV (SEQ ID NO: 27)
SP9	CYPTVTKGVSAACSKSSFIWLTGIIHPPNIGDQRALYHEIAKRPKVR DQEGRINV (SEQ ID NO: 28)
SP10	CYPTVTKGVSAACPESSFIWLTGIIHPPNIGDQKTLYHEIAKRPKVR DQEGRINV (SEQ ID NO: 29)
SP1	LGNPGTCYPGYKWNHTVTGVTASC SHAGKSSFYRNLLWLTGKNGS YPWGVHHPSNIGDQQTLYQTENAFTPEIAKRPKVRDQEGRMNYAN GNLIAPW (SEQ ID NO: 30)
SP2	LGNPGTCYPGYKWNHDVTGVSASC SHNGASSFYRNLLWLTKKNNL YPWGVHHPSTIADQQTLYHTENAFTPEIAIRPKVRDQEGRINYANGN LIAPW (SEQ ID NO: 31)

TABLE 9-continued

Engineered HA Antigenic Region Sequence	
SP3	LGNPGTCYPGYKWNHDSNGVTASCPHAGAKSFYRNLLWLVKKGN SYPWGVVHHPSTSADQQSLYQNANAFTPETIAIRPKVRDQEGRMNYAT GNLIAPW (SEQ ID NO: 32)
SP4	LGNPGTCYPGYKWNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGL YPWGVVHHPNIGDQRALYHTENAFTPETIAKRPKVRDQEGRINYANG NLIAPW (SEQ ID NO: 33)
SP5	LGNPGTCYPGYKWNHTVTGVSASCPHNGESSFYRNLLWLTGKNGL YPWGVVHHPNIGDQKTLYHTENAFTPETIAKRPKVRDQEGRINYANG NLIAPW (SEQ ID NO: 34)
SP6	LGNPGTCYPGDKWNHTVTKGVTAACSHAGKSSFYKNLIWLTGKNG SYPWGIHHPNIGDQQTLYQTEDTFKPEIAKRPKVRDQEGRMNYAN GNLVVPR (SEQ ID NO: 35)
SP7	LGNPGTCYPGDKWNHDVTKGVSAACSHNGASSFYKNLIWLTKKN NLYPWGIHHPSTIADQQTLYHTEDTFKPEIAIRPKVRDQEGRINYAN GNLVVPR (SEQ ID NO: 36)
SP8	LGNPGTCYPGDKWNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKG NSYPWGIHHPSTSADQQSLYQNADTFKPEIAIRPKVRDQEGRMNYA TGNLVVPR (SEQ ID NO: 37)
SP9	LGNPGTCYPGDKWNHTVTKGVSAACSHNGKSSFYKNLIWLTGKNG LYPWGIHHPNIGDQRALYHTEDTFKPEIAKRPKVRDQEGRINYAN GNLVVPR (SEQ ID NO: 38)
SP10	LGNPGTCYPGDKWNHTVTKGVSAACPHNGESSFYKNLIWLTGKNG LYPWGIHHPNIGDQKTLYHTEDTFKPEIAKRPKVRDQEGRINYANG NLVVPR (SEQ ID NO: 39)

Example 4. In Vivo Efficacy of Engineered Mosaic
Influenza B HA Polypeptides

This Example illustrates that engineered HA polypeptides made in accordance with the previous examples elicited immune responses in the form of broad antibody responses against several influenza B strains.

Immunization of Mice with rHAs

Soluble influenza B SMART HAs have been used to immunize mice. Mice were immunized three times (Day 0, Day 21, Day 42) with soluble rHA mixed 1:1 with AF03 (adjuvant) [the soluble proteins were used at the concentration and purity of the material produced on the PEPP system; no additional purification or concentration steps were performed]. 27 groups containing 3 mice each were tested as shown in Table 10.

TABLE 10

Group	Immunization n ¹ (Day 0, 21, 42)	Rationale
1	3 PBS	Negative Control
2	3 HA[br08_CH]	
3	3 HA[br08_CO1]	
4	3 HA[br08_DO1]	
5	3 HA[br08_DO2]	
6	3 HA[br08_DO3]	
7	3 HA[br08_RA65]	
8	3 HA[br08_RA8]	

TABLE 10-continued

Group	Immunization n ¹ (Day 0, 21, 42)	Rationale
9	3 HA[hk72_CH]	Positive Control
10	3 B/MASS/12 IIV	
11	3 HA[ma12_RA82]	
12	3 HA[pan90_DO2]	
13	3 HA[pan90_RA20]	
14	3 HA[sing79_DO1]	Wild-type
15	3 HA[sing79_RA103]	
17	3 HA[sing79_RA80]	
18	3 HA[yam88_DO1]	
19	3 HA[B_Lee_40]	
20	3 HA[B_HK_05_72]	
21	3 HA[B_Si_222_79]	
22	3 HA[B_Ya_16_88]	
23	3 HA[B_Pa_45_90]	
24	3 HA[B_Ma_2506_04]	
25	3 HA[B_Br_60_08]	
26	3 HA[B_Wi_01_10]	
27	3 HA[B_Ma_02_12]	
28	3 rHA[CA09]	CA/09 HA - Negative Control for HAI against B

Hemagglutination Inhibition (HAI) Assay

Terminal bleeds were taken on D56 and evaluated by HAI to a panel of Influenza B viruses. The immunogenicity data for these constructs are presented in FIG. 23 and Table 11.

5 of the 15 SMART HA constructs demonstrated some breadth of response against the Influenza B panel (Note: The current influenza B panel is limited and includes only a

single representative of the Victoria lineage; additionally the panel is biased to recent strains]. 3 of the 15 SMART HA constructs were cross-reactive to the 6 influenza B strains tested (both Yamagata and Victoria lineage). Similar breadth of response was only observed for the B/Massachusetts/ 2012 Inactivated vaccine (IIV); the use of B/Massachusetts/ 2012 recombinant HA was not cross-reactive to the Victoria lineage strain.

TABLE 11

	[HA]	B/Florida/ 4/2006	B/Bris/ 60/2008	B/Wisc/ 1/2010	B/Mass/ 2/2012	B/Texas/ 06/2011	B/Phuket/ 3073/2013	A/Calif/ 07/2009
PBS	0	<10	<10	<10	<10	<10	<10	<10
br08_CH	5.2	<10	320	20	<10	<10	<10	<10
br08_CO1	4.95	640	80	640	1280	160	320	<10
br08_DO1	7.075	<10	640	<10	<10	<10	<10	<10
br08_DO2	4.1	160	640	160	80	40	40	<10
br08_DO3	1.375	160	640	320	160	80	80	<10
br08_RA65	2.475	<10	160	<10	<10	<10	<10	<10
br08_RA8	8.025	<10	20	20	<10	<10	<10	<10
hk72_CH	5.25	<10	20	20	<10	<10	<10	<10
B/MA12_IIV	1.5	512	320	2560	5120	640	1280	<10
ma12_RA82	3.725	1280	20	640	640	160	160	<10
pan90_DO2	3.35	1280	20	2560	1280	640	1280	<10
pan90_RA20	5.35	20	20	40	<10	<10	<10	<10
sing79_DO1	2.05	<10	640	20	<10	<10	<10	<10
sing79_RA103	1.6	160	20	20	80	<10	<10	<10
sing79_RA80	3.1	<10	20	<10	<10	<10	<10	<10
yam88_DO1	1.15	<10	40	<10	<10	<10	<10	<10
B_Lee_40	2.125	<10	<10	<10	<10	<10	<10	<10
B_HK_05_72	1.575	20	20	20	<10	<10	<10	<10
B_Si_222_79	1.475	10	320	<10	<10	<10	<10	<10
B_Ya_16_88	1.025	1280	<10	640	1280	640	640	<10
B_Pa_45_90	4.1	40	<10	20	40	<10	<10	<10
B_Ma_2506_04	1.4	<10	640	<10	<10	<10	<10	<10
B_Br_60_08	3.425	640	5120	320	640	<10	80	<10
B_Wi_01_10	5.175	320	<10	640	160	160	160	<10
B_Ma_02_12	2.55	1280	<10	1280	1280	320	1280	<10
CA09_rHA	3	<10	<10	<10	<10	<10	<10	5120

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated that various alterations, modifications, and improvements will readily be apparent to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only and the invention is described in detail by the claims that follow.

EQUIVALENTS

Use of ordinal terms such as “first,” “second,” “third,” etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

The articles “a” and “an” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in,

employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or

process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, (e.g., in Markush group or similar format) it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. The publications, websites and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

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Xaa Xaa Xaa Xaa Gly Glu Xaa Xaa Xaa Xaa Gly Xaa Ile Ser Leu Xaa
35          40          45

Xaa Xaa Xaa Thr Lys Ser His Phe Xaa Asn Xaa Lys Xaa Thr Lys Xaa
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Xaa Xaa Lys Xaa Xaa Pro Xaa Xaa Pro Asn Cys Thr Asp Xaa Xaa Xaa
65          70          75          80

Xaa Xaa Xaa Xaa Pro Met Xaa Xaa Gly Thr Ile Pro Ser Ala Lys Xaa
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Xaa Xaa Xaa His Glu Val Arg Pro Val Thr Xaa Xaa Xaa Phe Xaa Xaa
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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly
115         120         125

Xaa Glu His Xaa Arg Xaa Xaa Thr His Asn Xaa Ile Asn Xaa Glu Xaa
130         135         140

Ala Pro Xaa Xaa Xaa Xaa Xaa Xaa Gly Thr Ser Gly Xaa Xaa Pro Asn
145         150         155         160

Ala Thr Xaa Lys Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp Ala Val Pro
165         170         175

Lys Xaa Asp Asn Asn Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
180         185         190

Xaa Xaa Ile Cys Ala Glu Gly Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
195         200         205

His Ser Asp Asp Lys Thr Gln Met Lys Xaa Xaa Xaa Xaa Xaa Xaa Xaa
210         215         220

Pro Xaa Lys Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
225         230         235         240

Xaa Xaa Xaa Xaa Asp Xaa Xaa Asn Xaa Thr Glu Xaa Xaa Xaa Xaa Xaa
245         250         255

Gln Ser Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Pro Gly Xaa
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Xaa Xaa Xaa Xaa Thr Xaa Gln Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
275         280         285

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305					310					315					320
Ser	Lys	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
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			340					345					350		
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370						375					380				
Xaa	Xaa	Thr	Xaa	Xaa	Xaa	Xaa	His	Xaa	Val	Ala	Val	Ala	Ala	Xaa	Leu
385					390					395					400
Lys	Xaa	Thr	Gln	Glu	Ala	Ile	Asn	Xaa	Ile	Thr	Lys	Asn	Leu	Asn	Xaa
				405					410					415	
Leu	Ser	Glu	Leu	Glu	Val	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
			420					425					430		
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		435					440					445			
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450						455				460					
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465					470				475						480
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				485				490						495	
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Xaa Xaa Xaa Xaa Gly Glu Xaa Xaa Xaa Xaa Gly Xaa Ile Pro Leu Xaa
35 40 45

Xaa Xaa Xaa Thr Lys Ser His Phe Xaa Asn Xaa Lys Xaa Thr Glu Xaa
50 55 60

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65 70 75 80

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Xaa Xaa Xaa His Glu Val Arg Pro Val Thr Xaa Xaa Xaa Phe Xaa Xaa
100 105 110

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly
115 120 125

Xaa Glu His Xaa Arg Xaa Xaa Thr Gln Asn Xaa Ile Asn Xaa Glu Xaa
130 135 140

Ala Pro Xaa Xaa Xaa Xaa Xaa Xaa Gly Thr Ser Gly Xaa Xaa Pro Asn
145 150 155 160

Ala Thr Xaa Lys Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp Ala Val Pro
165 170 175

Lys Xaa Asp Asn Asn Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

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210				215				220			
Pro	Xaa	Lys	Phe	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
225				230				235			
Xaa	Xaa	Xaa	Xaa	Gly	Xaa	Xaa	Asn	Thr	Glu	Xaa	Xaa
245				250				255			
Gln	Ser	Gly	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Lys	Pro
260				265				270			
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275				280				285			
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Ile	Gly	Glu	Ala	Asp	Xaa	Xaa	Xaa	Xaa	Lys	Tyr	Xaa
305				310				315			
Ser	Lys	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
325				330				335			
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340				345				350			
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355				360				365			
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370				375				380			
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385				390				395			
Lys	Xaa	Thr	Gln	Glu	Ala	Ile	Asn	Xaa	Ile	Thr	Lys
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Leu	Ser	Glu	Leu	Glu	Val	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
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450				455				460			
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465				470				475			
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
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Ile	Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Ala	Asn	Leu	Leu	Arg	
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Gly	Tyr	Glu	Asn	Ile	Arg	Leu	Ser	Thr	Gln	Asn	Val	Ile	Asp	Ala	Glu	
					115					120					125	
Lys	Ala	Pro	Gly	Gly	Pro	Tyr	Arg	Leu	Gly	Thr	Ser	Gly	Ser	Cys	Pro	
130					135					140						
Asn	Ala	Thr	Ser	Lys	Ser	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	
145					150					155					160	
Pro	Lys	Asp	Asn	Asn	Lys	Asn	Ala	Thr	Asn	Pro	Leu	Thr	Val	Glu	Val	
					165					170					175	
Pro	Tyr	Ile	Cys	Ala	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe	
					180					185					190	
His	Ser	Asp	Asp	Lys	Thr	Gln	Met	Lys	Asn	Leu	Tyr	Gly	Asp	Ser	Asn	
195					200					205						
Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val	
210					215					220						
Ser	Gln	Ile	Gly	Gly	Phe	Pro	Asp	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro	
225					230					235					240	
Gln	Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Met	Gln	Lys	Pro	Gly	Lys	
					245					250					255	
Thr	Gly	Thr	Ile	Val	Tyr	Gln	Arg	Gly	Val	Leu	Leu	Pro	Gln	Lys	Val	
					260					265					270	
Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu	
275					280					285						
Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys	
290					295					300						
Ser	Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys	
305					310					315					320	
Pro	Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr	
					325					330					335	
Arg	Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile	
					340					345					350	
Ala	Gly	Phe	Leu	Glu	Gly	Gly	Trp	Glu	Gly	Met	Ile	Ala	Gly	Trp	His	
355					360					365						
Gly	Tyr	Thr	Ser	His	Gly	Ala	His	Gly	Val	Ala	Val	Ala	Ala	Asp	Leu	
370					375					380						
Lys	Ser	Thr	Gln	Glu	Ala	Ile	Asn	Lys	Ile	Thr	Lys	Asn	Leu	Asn	Ser	
385					390					395					400	
Leu	Ser	Glu	Leu	Glu	Val	Lys	Asn	Leu	Gln	Arg	Leu	Ser	Gly	Ala	Met	
					405					410					415	
Asp	Glu	Leu	His	Asn	Glu	Ile	Leu	Glu	Leu	Asp	Glu	Lys	Val	Asp	Asp	
					420					425					430	
Leu	Arg	Ala	Asp	Thr	Ile	Ser	Ser	Gln	Ile	Glu	Leu	Ala	Val	Leu	Leu	
435					440					445						
Ser	Asn	Glu	Gly	Ile	Ile	Asn	Ser	Glu	Asp	Glu	His	Leu	Leu	Ala	Leu	
450					455					460						

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Glu	Arg	Lys	Leu	Lys	Lys	Met	Leu	Gly	Pro	Ser	Ala	Val	Asp	Ile	Gly	
465					470					475					480	
Asn	Gly	Cys	Phe	Glu	Thr	Lys	His	Lys	Cys	Asn	Gln	Thr	Cys	Leu	Asp	
				485					490					495		
Arg	Ile	Ala	Ala	Gly	Thr	Phe	Asn	Ala	Gly	Glu	Phe	Ser	Leu	Pro	Thr	
			500					505					510			
Phe	Asp	Ser	Leu	Asn	Ile	Thr	Ala	Ala	Ser	Leu	Asn	Asp	Asp	Gly	Leu	
		515					520					525				
Asp	Asn	His	Thr	Ile	Leu	Leu	Tyr	Tyr	Ser	Thr	Ala	Ala	Ser	Ser	Leu	
	530					535					540					
Ala	Val	Thr	Leu	Met	Leu	Ala	Ile	Phe	Ile	Val	Tyr	Met	Val	Ser	Arg	
545					550					555					560	
Asp	Asn	Val	Ser	Cys	Ser	Ile	Cys	Leu								
				565												
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<211> LENGTH: 568																
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Lys	Thr	Ala	Thr	Gln	Gly	Glu	Val	Asn	Val	Thr	Gly	Val	Ile	Pro	Leu	
			20					25					30			
Thr	Thr	Thr	Pro	Thr	Lys	Ser	His	Phe	Ala	Asn	Leu	Lys	Gly	Thr	Lys	
			35				40					45				
Thr	Arg	Gly	Lys	Leu	Cys	Pro	Asn	Cys	Leu	Asn	Cys	Thr	Asp	Leu	Asp	
	50					55					60					
Val	Ala	Leu	Gly	Arg	Pro	Met	Cys	Val	Gly	Thr	Thr	Pro	Ser	Ala	Lys	
65					70					75					80	
Ala	Ser	Ile	Leu	His	Glu	Val	Arg	Pro	Val	Thr	Ser	Gly	Cys	Phe	Pro	
				85					90					95		
Ile	Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Pro	Asn	Leu	Leu	Arg	
			100					105					110			
Gly	Tyr	Glu	Asn	Ile	Arg	Leu	Ser	Thr	Gln	Asn	Val	Ile	Asn	Ala	Glu	
		115				120						125				
Arg	Ala	Pro	Gly	Gly	Pro	Tyr	Arg	Leu	Gly	Thr	Ser	Gly	Ser	Cys	Pro	
	130					135					140					
Asn	Val	Thr	Ser	Arg	Asp	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	
145					150					155					160	
Pro	Arg	Asp	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Val	Glu	Val	Pro	
				165					170					175		
Tyr	Ile	Cys	Thr	Lys	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe	His	
			180					185					190			
Ser	Asp	Asn	Lys	Thr	Gln	Met	Lys	Asn	Leu	Tyr	Gly	Asp	Ser	Asn	Pro	
	195						200					205				
Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val	Ser	
	210					215					220					
Gln	Ile	Gly	Gly	Phe	Pro	Asn	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro	Gln	
225					230					235					240	
Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Pro	Gly	Lys	Thr	
				245					250					255		
Gly	Thr	Ile	Val	Tyr	Gln	Arg	Gly	Val	Leu	Leu	Pro	Gln	Lys	Val	Trp	

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260					265					270					
Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu	Ile
	275						280					285			
Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys	Ser
	290					295					300				
Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys	Pro
305					310					315					320
Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr	Arg
				325					330					335	
Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile	Ala
		340						345					350		
Gly	Phe	Leu	Glu	Gly	Gly	Trp	Glu	Gly	Met	Ile	Ala	Gly	Trp	His	Gly
	355						360					365			
Tyr	Thr	Ser	His	Gly	Ala	His	Gly	Val	Ala	Val	Ala	Ala	Asp	Leu	Lys
	370					375					380				
Ser	Thr	Gln	Glu	Ala	Ile	Asn	Lys	Ile	Thr	Lys	Asn	Leu	Asn	Ser	Leu
385					390					395					400
Ser	Glu	Leu	Glu	Val	Lys	Asn	Leu	Gln	Arg	Leu	Ser	Gly	Ala	Met	Asp
				405					410					415	
Glu	Leu	His	Asn	Glu	Ile	Leu	Glu	Leu	Asp	Glu	Lys	Val	Asp	Asp	Leu
			420					425					430		
Arg	Ala	Asp	Thr	Ile	Ser	Ser	Gln	Ile	Glu	Leu	Ala	Val	Leu	Leu	Ser
		435					440					445			
Asn	Glu	Gly	Ile	Ile	Asn	Ser	Glu	Asp	Glu	His	Leu	Leu	Ala	Leu	Glu
	450					455					460				
Arg	Lys	Leu	Lys	Lys	Met	Leu	Gly	Pro	Ser	Ala	Val	Asp	Ile	Gly	Asn
465					470					475					480
Gly	Cys	Phe	Glu	Thr	Lys	His	Lys	Cys	Asn	Gln	Thr	Cys	Leu	Asp	Arg
				485					490					495	
Ile	Ala	Ala	Gly	Thr	Phe	Asn	Ala	Gly	Glu	Phe	Ser	Leu	Pro	Thr	Phe
			500					505					510		
Asp	Ser	Leu	Asn	Ile	Thr	Ala	Ala	Ser	Leu	Asn	Asp	Asp	Gly	Leu	Asp
		515					520					525			
Asn	His	Thr	Ile	Leu	Leu	Tyr	Tyr	Ser	Thr	Ala	Ala	Ser	Ser	Leu	Ala
	530					535					540				
Val	Thr	Leu	Met	Ile	Ala	Ile	Phe	Ile	Val	Tyr	Met	Val	Ser	Arg	Asp
545					550					555					560
Asn	Val	Ser	Cys	Ser	Ile	Cys	Leu								
				565											
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<211> LENGTH: 570															
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Asp	Arg	Ile	Cys	Thr	Gly	Ile	Thr	Ser	Ser	Asn	Ser	Pro	His	Val	Val
1				5				10					15		
Lys	Thr	Ala	Thr	Gln	Gly	Glu	Val	Asn	Val	Thr	Gly	Val	Ile	Pro	Leu
		20						25					30		
Thr	Thr	Thr	Pro	Thr	Lys	Ser	His	Phe	Ala	Asn	Leu	Lys	Gly	Thr	Glu
		35					40					45			
Thr	Arg	Gly	Lys	Leu	Cys	Pro	Lys	Cys	Leu	Asn	Cys	Thr	Asp	Leu	Asp
	50					55					60				

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Val 65	Ala	Leu	Gly	Arg 70	Pro	Lys	Cys	Thr	Gly 75	Lys	Ile	Pro	Ser	Ala	Arg 80
Val	Ser	Ile	Leu	His 85	Glu	Val	Arg	Pro	Val 90	Thr	Ser	Gly	Cys	Phe	Pro 95
Ile	Met	His	Asp	Arg 100	Thr	Lys	Ile	Arg	Gln 105	Leu	Pro	Asn	Leu	Leu	Arg 110
Gly	Tyr	Glu	His	Ile	Arg	Leu	Ser	Thr	His	Asn	Val	Ile	Asn	Ala	Glu 115
Asn	Ala	Pro	Gly	Gly	Pro	Tyr	Lys	Ile	Gly	Thr	Ser	Gly	Ser	Cys	Pro 130
Asn	Ile	Thr	Asn	Gly	Asn	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val 145
Pro	Lys	Asn	Asp	Lys 165	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Ile	Glu 175
Val	Pro	Tyr	Ile	Cys 180	Thr	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly 190
Phe	His	Ser	Asp	Asp 195	Glu	Thr	Gln	Met	Ala	Lys	Leu	Tyr	Gly	Asp	Ser 205
Lys	Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr 210
Val	Ser	Gln	Ile	Gly	Gly	Phe	Pro	Asn	Gln	Thr	Glu	Asp	Gly	Gly	Leu 225
Pro	Gln	Ser	Gly	Arg 245	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Ser	Gly 255
Lys	Thr	Gly	Thr	Ile	Thr	Tyr	Gln	Arg	Gly	Ile	Leu	Leu	Pro	Gln	Lys 260
Val	Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro 275
Leu	Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn 290
Lys	Ser	Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn 305
Cys	Pro	Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys 325
Tyr	Arg	Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala 340
Ile	Ala	Gly	Phe	Leu	Glu	Gly	Gly	Trp	Glu	Gly	Met	Ile	Ala	Gly	Trp 355
His	Gly	Tyr	Thr	Ser	His	Gly	Ala	His	Gly	Val	Ala	Val	Ala	Ala	Asp 370
Leu	Lys	Ser	Thr	Gln	Glu	Ala	Ile	Asn	Lys	Ile	Thr	Lys	Asn	Leu	Asn 385
Ser	Leu	Ser	Glu	Leu	Glu	Val	Lys	Asn	Leu	Gln	Arg	Leu	Ser	Gly	Ala 405
Met	Asp	Glu	Leu	His	Asn	Glu	Ile	Leu	Glu	Leu	Asp	Glu	Lys	Val	Asp 420
Asp	Leu	Arg	Ala	Asp	Thr	Ile	Ser	Ser	Gln	Ile	Glu	Leu	Ala	Val	Leu 435
Leu	Ser	Asn	Glu	Gly	Ile	Ile	Asn	Ser	Glu	Asp	Glu	His	Leu	Leu	Ala 450
Leu	Glu	Arg	Lys	Leu	Lys	Lys	Met	Leu	Gly	Pro	Ser	Ala	Val	Glu	Ile 465
Gly	Asn	Gly	Cys	Phe	Glu	Thr	Lys	His	Lys	Cys	Asn	Gln	Thr	Cys	Leu 480

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485
Asp Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly Glu Phe Ser Leu Pro
500 505 510

Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly
515 520 525

Leu Asp Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser
530 535 540

Leu Ala Val Thr Leu Met Ile Ala Ile Phe Val Val Tyr Met Val Ser
545 550 555 560

Arg Asp Asn Val Ser Cys Ser Ile Cys Leu
565 570

<210> SEQ ID NO 10

<400> SEQUENCE: 10

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<210> SEQ ID NO 11
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 11

Gly Val Thr Ala Ser Ser Trp Leu Thr His His Pro Ser Asn Gly Asp
1 5 10 15

Gln Gln Thr Leu Lys Asp Gln Glu Gly Arg
20 25

<210> SEQ ID NO 12
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 12

Gly Val Ser Ala Ser Ser Trp Leu Thr His His Pro Ser Thr Ala Asp
1 5 10 15

Gln Gln Thr Leu Lys Asp Gln Glu Gly Arg
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<210> SEQ ID NO 13
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 13

Gly Val Thr Ala Ser Lys Trp Leu Val His His Pro Ser Thr Ala Asp
1 5 10 15

Gln Gln Ser Leu Lys Asp Gln Glu Gly Arg
20 25

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<210> SEQ ID NO 18
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
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Synthetic peptide"

<400> SEQUENCE: 18

Gly Val Ser Ala Ala Ser Trp Leu Thr His His Pro Pro Asn Gly Asp
1 5 10 15

Gln Arg Ala Leu Lys Asp Gln Glu Gly Arg
20 25

<210> SEQ ID NO 19

<211> LENGTH: 26

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<221> NAME/KEY: source
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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 19

Gly Val Ser Ala Ala Ser Trp Leu Thr His His Pro Pro Asn Gly Asp
1 5 10 15

Gln Lys Thr Leu Lys Asp Gln Glu Gly Arg
20 25

<210> SEQ ID NO 20

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<211> LENGTH: 54
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<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<221> NAME/KEY: source
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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 20

Cys Tyr Pro Thr Val Thr Gly Val Thr Ala Ser Cys Ser Lys Ser Ser
1 5 10 15

Phe Leu Trp Leu Thr Gly Val His His Pro Ser Asn Ile Gly Asp Gln
20 25 30

Gln Thr Leu Tyr Gln Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln
35 40 45

Glu Gly Arg Met Asn Ile
50

<210> SEQ ID NO 21

<211> LENGTH: 54

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<221> NAME/KEY: source
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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 21

Cys Tyr Pro Asp Val Thr Gly Val Ser Ala Ser Cys Ser Ala Ser Ser
1 5 10 15

Phe Leu Trp Leu Thr Lys Val His His Pro Ser Thr Ile Ala Asp Gln
20 25 30

Gln Thr Leu Tyr His Glu Ile Ala Ile Arg Pro Lys Val Arg Asp Gln
35 40 45

Glu Gly Arg Ile Asn Ile
50

<210> SEQ ID NO 22

<211> LENGTH: 54

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 22

Cys Tyr Pro Asp Ser Asn Gly Val Thr Ala Ser Cys Pro Ala Lys Ser
1 5 10 15

Phe Leu Trp Leu Val Lys Val His His Pro Ser Thr Ser Ala Asp Gln
20 25 30

Gln Ser Leu Tyr Gln Glu Ile Ala Ile Arg Pro Lys Val Arg Asp Gln
35 40 45

Glu Gly Arg Met Asn Ile
50

<210> SEQ ID NO 23
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 23

Cys Tyr Pro Thr Val Thr Gly Val Ser Ala Ser Cys Ser Lys Ser Ser
1 5 10 15

Phe Leu Trp Leu Thr Gly Val His His Pro Pro Asn Ile Gly Asp Gln
20 25 30

Arg Ala Leu Tyr His Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln
35 40 45

Glu Gly Arg Ile Asn Ile
50

<210> SEQ ID NO 24
<211> LENGTH: 54
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 24

Cys Tyr Pro Thr Val Thr Gly Val Ser Ala Ser Cys Pro Glu Ser Ser
1 5 10 15

Phe Leu Trp Leu Thr Gly Val His His Pro Pro Asn Ile Gly Asp Gln
20 25 30

Lys Thr Leu Tyr His Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln
35 40 45

Glu Gly Arg Ile Asn Ile
50

<210> SEQ ID NO 25
<211> LENGTH: 55
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

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<400> SEQUENCE: 25																			
Cys	Tyr	Pro	Thr	Val	Thr	Lys	Gly	Val	Thr	Ala	Ala	Cys	Ser	Lys	Ser				
1				5					10					15					
Ser	Phe	Ile	Trp	Leu	Thr	Gly	Ile	His	His	Pro	Ser	Asn	Ile	Gly	Asp				
			20					25					30						
Gln	Gln	Thr	Leu	Tyr	Gln	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Arg	Asp				
			35				40					45							
Gln	Glu	Gly	Arg	Met	Asn	Val													
	50				55														
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<212> TYPE: PRT																			
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<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"																			
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1				5					10					15					
Ser	Phe	Ile	Trp	Leu	Thr	Lys	Ile	His	His	Pro	Ser	Thr	Ile	Ala	Asp				
			20					25					30						
Gln	Gln	Thr	Leu	Tyr	His	Glu	Ile	Ala	Ile	Arg	Pro	Lys	Val	Arg	Asp				
			35				40					45							
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<211> LENGTH: 55																			
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<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"																			
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1				5					10					15					
Ser	Phe	Ile	Trp	Leu	Val	Lys	Ile	His	His	Pro	Ser	Thr	Ser	Ala	Asp				
			20					25					30						
Gln	Gln	Ser	Leu	Tyr	Gln	Glu	Ile	Ala	Ile	Arg	Pro	Lys	Val	Arg	Asp				
			35				40					45							
Gln	Glu	Gly	Arg	Met	Asn	Val													
	50				55														
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<211> LENGTH: 55																			
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1				5					10					15					
Ser	Phe	Ile	Trp	Leu	Thr	Gly	Ile	His	His	Pro	Pro	Asn	Ile	Gly	Asp				
			20					25					30						

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Gln	Arg	Ala	Leu	Tyr	His	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Arg	Asp
	35						40					45			
Gln	Glu	Gly	Arg	Ile	Asn	Val									
	50					55									
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<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"															
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Cys	Tyr	Pro	Thr	Val	Thr	Lys	Gly	Val	Ser	Ala	Ala	Cys	Pro	Glu	Ser
1				5					10					15	
Ser	Phe	Ile	Trp	Leu	Thr	Gly	Ile	His	His	Pro	Pro	Asn	Ile	Gly	Asp
			20					25					30		
Gln	Lys	Thr	Leu	Tyr	His	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Arg	Asp
	35						40					45			
Gln	Glu	Gly	Arg	Ile	Asn	Val									
	50					55									
<210> SEQ ID NO 30															
<211> LENGTH: 97															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<221> NAME/KEY: source															
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"															
<400> SEQUENCE: 30															
Leu	Gly	Asn	Pro	Gly	Thr	Cys	Tyr	Pro	Gly	Tyr	Lys	Trp	Asn	His	Thr
1				5					10					15	
Val	Thr	Gly	Val	Thr	Ala	Ser	Cys	Ser	His	Ala	Gly	Lys	Ser	Ser	Phe
			20					25					30		
Tyr	Arg	Asn	Leu	Leu	Trp	Leu	Thr	Gly	Lys	Asn	Gly	Ser	Tyr	Pro	Trp
	35						40					45			
Gly	Val	His	His	Pro	Ser	Asn	Ile	Gly	Asp	Gln	Gln	Thr	Leu	Tyr	Gln
	50					55				60					
Thr	Glu	Asn	Ala	Phe	Thr	Pro	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Arg
65					70				75					80	
Asp	Gln	Glu	Gly	Arg	Met	Asn	Tyr	Ala	Asn	Gly	Asn	Leu	Ile	Ala	Pro
				85					90					95	
Trp															
<210> SEQ ID NO 31															
<211> LENGTH: 97															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<221> NAME/KEY: source															
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"															
<400> SEQUENCE: 31															
Leu	Gly	Asn	Pro	Gly	Thr	Cys	Tyr	Pro	Gly	Tyr	Lys	Trp	Asn	His	Asp
1				5					10					15	
Val	Thr	Gly	Val	Ser	Ala	Ser	Cys	Ser	His	Asn	Gly	Ala	Ser	Ser	Phe

[illegible]

<400> SEQUENCE: 32

Tyr Arg Asn Leu Leu Trp Leu Val Lys Lys Gly Asn Ser Tyr Pro Trp
35 40 45

Asn Ala Asn Ala Phe Thr Pro Glu Ile Ala Ile Arg Pro Lys Val Arg
65 70 75 80

Asp Gln Glu Gly Arg Met Asn Tyr Ala Thr Gly Asn Leu Ile Ala Pro
85 90 95

Trp

<400> SEQUENCE: 33

Leu Gly Asn Pro Gly Thr Cys Tyr Pro Gly Tyr Lys Trp Asn His Thr
1 5 10 15

Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe
20 25 30

Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Trp
35 40 45

Gly Val His His Pro Pro Asn Ile Gly Asp Gln Arg Ala Leu Tyr His
50 55 60

Thr Glu Asn Ala Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg
65 70 75 80

Asp Gln Glu Gly Arg Ile Asn Tyr Ala Asn Gly Asn Leu Ile Ala Pro
85 90 95

Trp

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<210> SEQ ID NO 34
<211> LENGTH: 97
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 34

Leu Gly Asn Pro Gly Thr Cys Tyr Pro Gly Tyr Lys Trp Asn His Thr
1 5 10 15

Val Thr Gly Val Ser Ala Ser Cys Pro His Asn Gly Glu Ser Ser Phe
20 25 30

Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Trp
35 40 45

Gly Val His His Pro Pro Asn Ile Gly Asp Gln Lys Thr Leu Tyr His
50 55 60

Thr Glu Asn Ala Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg
65 70 75 80

Asp Gln Glu Gly Arg Ile Asn Tyr Ala Asn Gly Asn Leu Ile Ala Pro
85 90 95

Trp

<210> SEQ ID NO 35
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 35

Leu Gly Asn Pro Gly Thr Cys Tyr Pro Gly Asp Lys Trp Asn His Thr
1 5 10 15

Val Thr Lys Gly Val Thr Ala Ala Cys Ser His Ala Gly Lys Ser Ser
20 25 30

Phe Tyr Lys Asn Leu Ile Trp Leu Thr Gly Lys Asn Gly Ser Tyr Pro
35 40 45

Trp Gly Ile His His Pro Ser Asn Ile Gly Asp Gln Gln Thr Leu Tyr
50 55 60

Gln Thr Glu Asp Thr Phe Lys Pro Glu Ile Ala Lys Arg Pro Lys Val
65 70 75 80

Arg Asp Gln Glu Gly Arg Met Asn Tyr Ala Asn Gly Asn Leu Val Val
85 90 95

Pro Arg

<210> SEQ ID NO 36
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 36

Leu Gly Asn Pro Gly Thr Cys Tyr Pro Gly Asp Lys Trp Asn His Asp
1 5 10 15

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Val	Thr	Lys	Gly	Val	Ser	Ala	Ala	Cys	Ser	His	Asn	Gly	Ala	Ser	Ser	
			20					25					30			
Phe	Tyr	Lys	Asn	Leu	Ile	Trp	Leu	Thr	Lys	Lys	Asn	Asn	Leu	Tyr	Pro	
		35					40					45				
Trp	Gly	Ile	His	His	Pro	Ser	Thr	Ile	Ala	Asp	Gln	Gln	Thr	Leu	Tyr	
	50					55					60					
His	Thr	Glu	Asp	Thr	Phe	Lys	Pro	Glu	Ile	Ala	Ile	Arg	Pro	Lys	Val	
65					70					75					80	
Arg	Asp	Gln	Glu	Gly	Arg	Ile	Asn	Tyr	Ala	Asn	Gly	Asn	Leu	Val	Val	
			85						90					95		
Pro	Arg															
<210> SEQ ID NO 37																
<211> LENGTH: 98																
<212> TYPE: PRT																
<213> ORGANISM: Artificial Sequence																
<220> FEATURE:																
<221> NAME/KEY: source																
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"																
<400> SEQUENCE: 37																
Leu	Gly	Asn	Pro	Gly	Thr	Cys	Tyr	Pro	Gly	Asp	Lys	Trp	Asn	His	Asp	
1				5					10					15		
Ser	Asn	Lys	Gly	Val	Thr	Ala	Ala	Cys	Pro	His	Ala	Gly	Ala	Lys	Ser	
			20					25					30			
Phe	Tyr	Lys	Asn	Leu	Ile	Trp	Leu	Val	Lys	Lys	Gly	Asn	Ser	Tyr	Pro	
		35					40					45				
Trp	Gly	Ile	His	His	Pro	Ser	Thr	Ser	Ala	Asp	Gln	Gln	Ser	Leu	Tyr	
	50					55					60					
Gln	Asn	Ala	Asp	Thr	Phe	Lys	Pro	Glu	Ile	Ala	Ile	Arg	Pro	Lys	Val	
65					70					75					80	
Arg	Asp	Gln	Glu	Gly	Arg	Met	Asn	Tyr	Ala	Thr	Gly	Asn	Leu	Val	Val	
			85						90					95		
Pro	Arg															
<210> SEQ ID NO 38																
<211> LENGTH: 98																
<212> TYPE: PRT																
<213> ORGANISM: Artificial Sequence																
<220> FEATURE:																
<221> NAME/KEY: source																
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"																
<400> SEQUENCE: 38																
Leu	Gly	Asn	Pro	Gly	Thr	Cys	Tyr	Pro	Gly	Asp	Lys	Trp	Asn	His	Thr	
1				5					10					15		
Val	Thr	Lys	Gly	Val	Ser	Ala	Ala	Cys	Ser	His	Asn	Gly	Lys	Ser	Ser	
			20					25					30			
Phe	Tyr	Lys	Asn	Leu	Ile	Trp	Leu	Thr	Gly	Lys	Asn	Gly	Leu	Tyr	Pro	
		35					40					45				
Trp	Gly	Ile	His	His	Pro	Pro	Asn	Ile	Gly	Asp	Gln	Arg	Ala	Leu	Tyr	
	50					55					60					
His	Thr	Glu	Asp	Thr	Phe	Lys	Pro	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	
65					70					75					80	
Arg	Asp	Gln	Glu	Gly	Arg	Ile	Asn	Tyr	Ala	Asn	Gly	Asn	Leu	Val	Val	
			85						90					95		

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Pro Arg

<210> SEQ ID NO 39
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 39

Leu Gly Asn Pro Gly Thr Cys Tyr Pro Gly Asp Lys Trp Asn His Thr
1 5 10 15
Val Thr Lys Gly Val Ser Ala Ala Cys Pro His Asn Gly Glu Ser Ser
20 25 30
Phe Tyr Lys Asn Leu Ile Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro
35 40 45
Trp Gly Ile His His Pro Pro Asn Ile Gly Asp Gln Lys Thr Leu Tyr
50 55 60
His Thr Glu Asp Thr Phe Lys Pro Glu Ile Ala Lys Arg Pro Lys Val
65 70 75 80
Arg Asp Gln Glu Gly Arg Ile Asn Tyr Ala Asn Gly Asn Leu Val Val
85 90 95

Pro Arg

<210> SEQ ID NO 40
<211> LENGTH: 585
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 40

Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
1 5 10 15
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
20 25 30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr
35 40 45
Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu Thr
50 55 60
Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp Val
65 70 75 80
Ala Leu Gly Arg Pro Lys Cys Thr Gly Lys Ile Pro Ser Ala Arg Val
85 90 95
Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile
100 105 110
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly
115 120 125
Tyr Glu His Ile Arg Leu Ser Thr Gln Asn Val Ile Asn Ala Glu Asn
130 135 140
Ala Pro Gly Gly Pro Tyr Lys Ile Gly Thr Ser Gly Ser Cys Pro Asn
145 150 155 160
Ala Thr Asn Lys Ser Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro
165 170 175

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Lys	Asn	Asp	Asn	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Ile	Glu	Val	
			180					185					190			
Pro	Tyr	Ile	Cys	Thr	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe	
		195					200					205				
His	Ser	Asp	Asn	Lys	Thr	Gln	Met	Lys	Lys	Leu	Tyr	Gly	Asp	Ser	Lys	
	210					215					220					
Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val	
225					230					235					240	
Ser	Gln	Ile	Gly	Gly	Phe	Pro	Asn	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro	
			245						250					255		
Gln	Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Pro	Gly	Lys	
			260					265					270			
Thr	Gly	Thr	Ile	Val	Tyr	Gln	Arg	Gly	Ile	Leu	Leu	Pro	Gln	Lys	Val	
		275					280						285			
Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu	
	290						295				300					
Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys	
305					310					315					320	
Ser	Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys	
				325					330					335		
Pro	Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr	
			340					345					350			
Arg	Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile	
		355					360					365				
Ala	Gly	Phe	Leu	Glu	Gly	Gly	Trp	Glu	Gly	Met	Ile	Ala	Gly	Trp	His	
	370					375					380					
Gly	Tyr	Thr	Ser	His	Gly	Ala	His	Gly	Val	Ala	Val	Ala	Ala	Asp	Leu	
385					390					395					400	
Lys	Ser	Thr	Gln	Glu	Ala	Ile	Asn	Lys	Ile	Thr	Lys	Asn	Leu	Asn	Ser	
			405						410					415		
Leu	Ser	Glu	Leu	Glu	Val	Lys	Asn	Leu	Gln	Arg	Leu	Ser	Gly	Ala	Met	
		420						425					430			
Asp	Glu	Leu	His	Asn	Glu	Ile	Leu	Glu	Leu	Asp	Glu	Lys	Val	Asp	Asp	
		435					440					445				
Leu	Arg	Ala	Asp	Thr	Ile	Ser	Ser	Gln	Ile	Glu	Leu	Ala	Val	Leu	Leu	
	450					455					460					
Ser	Asn	Glu	Gly	Ile	Ile	Asn	Ser	Glu	Asp	Glu	His	Leu	Leu	Ala	Leu	
465					470					475					480	
Glu	Arg	Lys	Leu	Lys	Lys	Met	Leu	Gly	Pro	Ser	Ala	Val	Glu	Ile	Gly	
			485						490					495		
Asn	Gly	Cys	Phe	Glu	Thr	Lys	His	Lys	Cys	Asn	Gln	Thr	Cys	Leu	Asp	
		500						505					510			
Arg	Ile	Ala	Ala	Gly	Thr	Phe	Asp	Ala	Gly	Glu	Phe	Ser	Leu	Pro	Thr	
		515					520					525				
Phe	Asp	Ser	Leu	Asn	Ile	Thr	Ala	Ala	Ser	Leu	Asn	Asp	Asp	Gly	Leu	
	530					535					540					
Asp	Asn	His	Thr	Ile	Leu	Leu	Tyr	Tyr	Ser	Thr	Ala	Ala	Ser	Ser	Leu	
545					550					555					560	
Ala	Val	Thr	Leu	Met	Ile	Ala	Ile	Phe	Val	Val	Tyr	Met	Val	Ser	Arg	
			565					570					575			
Asp	Asn	Val	Ser	Cys	Ser	Ile	Cys	Leu								
		580						585								

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<210> SEQ ID NO 41																			
<211> LENGTH: 585																			
<212> TYPE: PRT																			
<213> ORGANISM: Artificial Sequence																			
<220> FEATURE:																			
<221> NAME/KEY: source																			
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"																			
<400> SEQUENCE: 41																			
Met	Lys	Ala	Ile	Ile	Val	Leu	Leu	Met	Val	Val	Thr	Ser	Asn	Ala	Asp				
1				5					10					15					
Arg	Ile	Cys	Thr	Gly	Ile	Thr	Ser	Ser	Asn	Ser	Pro	His	Val	Val	Lys				
			20				25						30						
Thr	Ala	Thr	Gln	Gly	Glu	Val	Asn	Val	Thr	Gly	Val	Ile	Pro	Leu	Thr				
			35				40					45							
Thr	Thr	Pro	Thr	Lys	Ser	Tyr	Phe	Ala	Asn	Leu	Lys	Gly	Thr	Glu	Thr				
	50					55					60								
Arg	Gly	Lys	Leu	Cys	Pro	Lys	Cys	Leu	Asn	Cys	Thr	Asp	Leu	Asp	Val				
65					70				75					80					
Ala	Leu	Gly	Arg	Pro	Lys	Cys	Thr	Gly	Lys	Ile	Pro	Ser	Ala	Lys	Val				
				85					90					95					
Ser	Ile	Leu	His	Glu	Val	Arg	Pro	Val	Thr	Ser	Gly	Cys	Phe	Pro	Ile				
			100					105					110						
Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Pro	Asn	Leu	Leu	Arg	Gly				
		115					120					125							
Tyr	Glu	His	Ile	Arg	Leu	Ser	Thr	Gln	Asn	Val	Ile	Asp	Ala	Glu	Asn				
	130					135					140								
Ala	Pro	Gly	Gly	Pro	Tyr	Lys	Ile	Gly	Thr	Ser	Gly	Ser	Cys	Pro	Asn				
145					150					155				160					
Ala	Thr	Asn	Lys	Ser	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro				
				165					170				175						
Lys	Asn	Asp	Asn	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Ile	Glu	Val				
		180						185					190						
Pro	Tyr	Ile	Cys	Thr	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe				
	195					200						205							
His	Ser	Asp	Asn	Lys	Thr	Gln	Met	Lys	Lys	Leu	Tyr	Gly	Asp	Ser	Lys				
	210					215					220								
Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val				
225					230					235				240					
Ser	Gln	Ile	Gly	Gly	Phe	Pro	Asp	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro				
			245						250				255						
Gln	Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Pro	Gly	Lys				
		260					265					270							
Thr	Gly	Thr	Ile	Val	Tyr	Gln	Arg	Gly	Ile	Leu	Leu	Pro	Gln	Lys	Val				
	275					280						285							
Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu				
	290					295					300								
Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys				
305					310					315				320					
Ser	Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys				
			325						330				335						
Pro	Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr				
		340					345					350							
Arg	Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile				

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355					360					365							
Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Val Ala Gly Trp His																	
370						375						380					
Gly Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu																	
385						390						395					
Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser																	
					405						410						
Leu Ser Glu Leu Glu Ile Lys Asn Leu Gln Arg Leu Ser Gly Ala Met																	
					420						425						
Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp																	
					435						440						
Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu																	
					450						455						
Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu																	
465						470						475					
Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Glu Ile Gly																	
					485						490						
Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp																	
					500						505						
Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly Glu Phe Ser Leu Pro Thr																	
					515						520						
Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu																	
					530						535						
Asp Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu																	
					545						550						
Ala Val Thr Leu Met Ile Ala Ile Phe Val Val Tyr Met Val Ser Arg																	
					565						570						
Asp Asn Val Ser Cys Ser Ile Cys Leu																	
					580						585						
<210> SEQ ID NO 42																	
<211> LENGTH: 585																	
<212> TYPE: PRT																	
<213> ORGANISM: Artificial Sequence																	
<220> FEATURE:																	
<221> NAME/KEY: source																	
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"																	
<400> SEQUENCE: 42																	
Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp																	
1						5						10					
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys																	
					20						25						
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Ser Leu Thr																	
					35						40						
Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Lys Thr																	
					50						55						
Arg Gly Lys Leu Cys Pro Lys Cys Pro Asn Cys Thr Asp Leu Asp Val																	
					65						70						
Ala Leu Gly Arg Pro Met Cys Thr Gly Thr Ile Pro Ser Ala Lys Val																	
					85						90						
Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile																	
					100						105						
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly																	
					115						120						

Tyr	Glu	His	Ile	Arg	Leu	Ser	Thr	His	Asn	Val	Ile	Asn	Ala	Glu	Asn
130						135					140				
Ala	Pro	Gly	Gly	Pro	Tyr	Lys	Ile	Gly	Thr	Ser	Gly	Ser	Cys	Pro	Asn
145					150					155					160
Ala	Thr	Asn	Lys	Ile	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro
				165					170					175	
Lys	Asn	Asp	Asn	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Ile	Glu	Val
			180					185					190		
Pro	Tyr	Ile	Cys	Ala	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe
		195					200					205			
His	Ser	Asp	Asp	Lys	Thr	Gln	Met	Lys	Lys	Leu	Tyr	Gly	Asp	Ser	Lys
	210					215					220				
Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val
225					230					235					240
Ser	Gln	Ile	Gly	Asp	Phe	Pro	Asn	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro
				245					250					255	
Gln	Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Pro	Gly	Lys
			260					265					270		
Thr	Gly	Thr	Ile	Thr	Tyr	Gln	Arg	Gly	Ile	Leu	Leu	Pro	Gln	Lys	Val
		275					280					285			
Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu
	290					295					300				
Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys
305					310					315					320
Ser	Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys
				325					330					335	
Pro	Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr
			340					345					350		
Arg	Pro	Pro	Thr	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile
		355					360					365			
Ala	Gly	Phe	Leu	Glu	Gly	Gly	Trp	Glu	Gly	Met	Ile	Ala	Gly	Trp	His
	370					375					380				
Gly	Tyr	Thr	Ser	His	Gly	Ala	His	Gly	Val	Ala	Val	Ala	Ala	Asp	Leu
385					390					395					400
Lys	Ser	Thr	Gln	Glu	Ala	Ile	Asn	Lys	Ile	Thr	Lys	Asn	Leu	Asn	Ser
				405					410					415	
Leu	Ser	Glu	Leu	Glu	Val	Lys	Asn	Leu	Gln	Arg	Leu	Ser	Gly	Ala	Met
			420					425					430		
Asp	Glu	Leu	His	Asn	Glu	Ile	Leu	Glu	Leu	Asp	Glu	Lys	Val	Asp	Asp
		435					440					445			
Leu	Arg	Ala	Asp	Thr	Ile	Ser	Ser	Gln	Ile	Glu	Leu	Ala	Val	Leu	Leu
		450				455					460				
Ser	Asn	Glu	Gly	Ile	Ile	Asn	Ser	Glu	Asp	Glu	His	Leu	Leu	Ala	Leu
465					470					475					480
Glu	Arg														

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Asp	Asn	His	Thr	Ile	Leu	Leu	Tyr	Tyr	Ser	Thr	Ala	Ala	Ser	Ser	Leu
545					550					555					560
Ala	Val	Thr	Leu	Met	Ile	Ala	Ile	Phe	Val	Val	Tyr	Met	Val	Ser	Arg
				565					570					575	
Asp	Asn	Val	Ser	Cys	Ser	Ile	Cys	Leu							
			580					585							
<210> SEQ ID NO 43															
<211> LENGTH: 585															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<221> NAME/KEY: source															
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"															
<400> SEQUENCE: 43															
Met	Lys	Ala	Ile	Ile	Val	Leu	Leu	Met	Val	Val	Thr	Ser	Asn	Ala	Asp
1				5					10					15	
Arg	Ile	Cys	Thr	Gly	Ile	Thr	Ser	Ser	Asn	Ser	Pro	His	Val	Val	Lys
			20					25					30		
Thr	Ala	Thr	Gln	Gly	Glu	Val	Asn	Val	Thr	Gly	Val	Ile	Pro	Leu	Thr
			35				40					45			
Thr	Thr	Pro	Thr	Lys	Ser	Tyr	Phe	Ala	Asn	Leu	Lys	Gly	Thr	Glu	Thr
	50					55					60				
Arg	Gly	Lys	Leu	Cys	Pro	Asn	Cys	Leu	Asn	Cys	Thr	Asp	Leu	Asp	Val
65					70					75					80
Ala	Leu	Gly	Arg	Pro	Lys	Cys	Val	Gly	Lys	Ile	Pro	Ser	Ala	Lys	Ala
				85					90					95	
Ser	Ile	Leu	His	Glu	Val	Arg	Pro	Val	Thr	Ser	Gly	Cys	Phe	Pro	Ile
			100					105					110		
Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Pro	Asn	Leu	Leu	Arg	Gly
		115					120					125			
Tyr	Glu	His	Ile	Arg	Leu	Ser	Thr	Gln	Asn	Val	Ile	Asp	Ala	Glu	Arg
	130					135					140				
Ala	Pro	Gly	Gly	Pro	Tyr	Arg	Leu	Gly	Thr	Ser	Gly	Ser	Cys	Pro	Asn
145					150					155					160
Ala	Thr	Ser	Lys	Ser	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro
				165					170					175	
Lys	Asp	Asp	Asn	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Val	Glu	Val
			180					185					190		
Pro	Tyr	Ile	Cys	Thr	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe
		195					200					205			
His	Ser	Asp	Asn	Lys	Thr	Gln	Met	Lys	Asn	Leu	Tyr	Gly	Asp	Ser	Asn
	210					215					220				
Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val
225					230					235					240
Ser	Gln	Ile	Gly	Gly	Phe	Pro	Asp	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro
			245						250					255	
Gln	Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Pro	Gly	Lys
			260					265					270		
Thr	Gly	Thr	Ile	Val	Tyr	Gln	Arg	Gly	Val	Leu	Leu	Pro	Gln	Lys	Val
		275					280					285			
Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu
	290					295					300				
Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys

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305	310	315	320
Ser Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys	325	330	335
Pro Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr	340	345	350
Arg Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile	355	360	365
Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Val Ala Gly Trp His	370	375	380
Gly Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu	385	390	395
Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser	405	410	415
Leu Ser Glu Leu Glu Ile Lys Asn Leu Gln Arg Leu Ser Gly Ala Met	420	425	430
Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp	435	440	445
Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu	450	455	460
Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu	465	470	475
Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly	485	490	495
Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp	500	505	510
Arg Ile Ala Ala Gly Thr Phe Asn Ala Gly Glu Phe Ser Leu Pro Thr	515	520	525
Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu	530	535	540
Asp Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu	545	550	555
Ala Val Thr Leu Met Ile Ala Ile Phe Ile Val Tyr Met Val Ser Arg	565	570	575
Asp Asn Val Ser Cys Ser Ile Cys Leu	580	585	
<210> SEQ ID NO 44			
<211> LENGTH: 585			
<212> TYPE: PRT			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<221> NAME/KEY: source			
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"			
<400> SEQUENCE: 44			
Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp	1	5	10
Arg Ile Cys Thr Gly Ile Thr Ser Ser Lys Ser Pro His Val Val Lys	20	25	30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr	35	40	45
Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Arg Gly Thr Lys Thr	50	55	60
Arg Gly Lys Leu Cys Pro Asp Cys Leu Asn Cys Thr Asp Leu Asp Val	65	70	75
			80

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Ala	Leu	Gly	Arg	Pro	Lys	Cys	Val	Gly	Asn	Thr	Pro	Ser	Ala	Lys	Ala		
				85					90					95			
Ser	Ile	Leu	His	Glu	Val	Arg	Pro	Val	Thr	Ser	Gly	Cys	Phe	Pro	Ile		
			100					105					110				
Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Ala	Asn	Leu	Leu	Arg	Gly		
		115					120					125					
Tyr	Glu	His	Ile	Arg	Leu	Ser	Asn	Tyr	Asn	Val	Ile	Asp	Ala	Glu	Lys		
	130					135					140						
Ala	Pro	Gly	Gly	Pro	Tyr	Arg	Leu	Gly	Thr	Ser	Arg	Ser	Cys	Pro	Asn		
145					150					155					160		
Val	Thr	Ser	Arg	Ser	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro		
				165					170					175			
Lys	Asp	Asp	Ser	Asn	Lys	Asn	Ala	Thr	Asn	Pro	Leu	Thr	Val	Glu	Val		
			180					185					190				
Pro	Tyr	Ile	Cys	Thr	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe		
		195					200					205					
His	Ser	Asp	Asn	Lys	Thr	Gln	Met	Val	Asn	Leu	Tyr	Gly	Asp	Ser	Asn		
	210					215					220						
Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val		
225					230					235					240		
Ser	Gln	Ile	Gly	Asp	Phe	Pro	Asn	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro		
			245						250					255			
Gln	Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Met	Gln	Lys	Ser	Gly	Lys		
		260						265					270				
Thr	Gly	Thr	Ile	Thr	Tyr	Gln	Arg	Gly	Val	Leu	Leu	Pro	Gln	Lys	Val		
		275				280						285					
Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Thr	Leu	Pro	Leu		
	290					295					300						
Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys		
305					310					315					320		
Ser	Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys		
			325						330					335			
Pro	Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr		
		340						345					350				
Arg	Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile		
		355					360					365					
Ala	Gly	Phe	Leu	Glu	Gly	Gly	Trp	Glu	Gly	Met	Ile	Ala	Gly	Trp	His		
	370					375					380						
Gly	Tyr	Thr	Ser	His	Gly	Ala	His	Gly	Val	Ala	Val	Ala	Ala	Asp	Leu		
385					390					395					400		
Lys	Ser	Thr	Gln	Glu	Ala	Ile	Asn	Lys	Ile	Thr	Lys	Asn	Leu	Asn	Ser		
			405						410					415			
Leu	Ser	Glu	Leu	Glu	Val	Lys	Asn	Leu	Gln	Arg	Leu	Ser	Gly	Ala	Met		
		420						425					430				
Asp	Glu	Leu	His	Asn	Glu	Ile	Leu	Glu	Leu	Asp	Glu	Lys	Val	Asp	Asp		
	435					440					445						
Leu	Arg	Ala	Asp	Thr	Ile	Ser	Ser	Gln	Ile	Glu	Leu	Ala	Val	Leu	Leu		
	450					455					460						
Ser	Asn	Glu	Gly	Ile	Ile	Asn	Ser	Glu	Asp	Glu	His	Leu	Leu	Ala	Leu		
465					470				475						480		
Glu	Arg	Lys	Leu	Lys	Lys	Met	Leu	Gly	Pro	Ser	Ala	Val	Asp	Ile	Gly		
			485					490						495			

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Asn	Gly	Cys	Phe	Glu	Thr	Lys	His	Lys	Cys	Asn	Gln	Thr	Cys	Leu	Asp	
			500					505					510			
Arg	Ile	Ala	Ala	Gly	Thr	Phe	Asn	Ala	Gly	Glu	Phe	Ser	Leu	Pro	Thr	
		515					520					525				
Phe	Asp	Ser	Leu	Asn	Ile	Thr	Ala	Ala	Ser	Leu	Asn	Asp	Asp	Gly	Leu	
	530					535					540					
Asp	Asn	His	Thr	Ile	Leu	Leu	Tyr	Tyr	Ser	Thr	Ala	Ala	Ser	Ser	Leu	
545					550					555					560	
Ala	Val	Thr	Leu	Met	Leu	Ala	Ile	Phe	Ile	Val	Tyr	Met	Val	Ser	Arg	
				565					570					575		
Asp	Asn	Val	Ser	Cys	Ser	Ile	Cys	Leu								
			580					585								
<210> SEQ ID NO 45																
<211> LENGTH: 585																
<212> TYPE: PRT																
<213> ORGANISM: Artificial Sequence																
<220> FEATURE:																
<221> NAME/KEY: source																
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"																
<400> SEQUENCE: 45																
Met	Lys	Ala	Ile	Ile	Val	Leu	Leu	Met	Val	Val	Thr	Ser	Asn	Ala	Asp	
1				5					10					15		
Arg	Ile	Cys	Thr	Gly	Ile	Thr	Ser	Ser	Asn	Ser	Pro	His	Val	Val	Lys	
			20					25					30			
Thr	Ala	Thr	Gln	Gly	Glu	Val	Asn	Val	Thr	Gly	Val	Ile	Pro	Leu	Thr	
			35				40					45				
Thr	Thr	Pro	Thr	Lys	Ser	Tyr	Phe	Ala	Asn	Leu	Lys	Gly	Thr	Lys	Thr	
	50					55					60					
Arg	Gly	Lys	Leu	Cys	Pro	Asn	Cys	Leu	Asn	Cys	Thr	Asp	Leu	Asp	Val	
65					70					75					80	
Ala	Leu	Gly	Arg	Pro	Met	Cys	Met	Gly	Thr	Ile	Pro	Ser	Ala	Lys	Ala	
				85					90					95		
Ser	Ile	Leu	His	Glu	Val	Arg	Pro	Val	Thr	Ser	Gly	Cys	Phe	Pro	Ile	
			100					105					110			
Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Pro	Asn	Leu	Leu	Arg	Gly	
		115					120					125				
Tyr	Glu	Asn	Ile	Arg	Leu	Ser	Thr	His	Asn	Val	Ile	Asn	Ala	Glu	Arg	
	130					135					140					
Ala	Pro	Gly	Gly	Pro	Tyr	Ile	Ile	Gly	Thr	Ser	Gly	Ser	Cys	Pro	Asn	
145					150					155					160	
Ala	Thr	Asn	Lys	Asn	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro	
				165					170					175		
Lys	Asp	Asp	Asn	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Val	Glu	Val	
			180					185					190			
Pro	Tyr	Ile	Cys	Thr	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe	
	195						200					205				
His	Ser	Asp	Asn	Lys	Thr	Gln	Met	Lys	Lys	Leu	Tyr	Gly	Asp	Ser	Lys	
	210						215					220				
Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val	
225					230					235					240	
Ser	Gln	Ile	Gly	Gly	Phe	Pro	Asp	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro	
			245						250					255		
Gln	Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Ser	Gly	Lys	

260					265					270						
Thr	Gly	Thr	Ile	Thr	Tyr	Gln	Arg	Gly	Val	Leu	Leu	Pro	Gln	Lys	Val	
275					280					285						
Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu	
290					295					300						
Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys	
305					310					315					320	
Ser	Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys	
325					330					335						
Pro	Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr	
340					345					350						
Arg	Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile	
355					360					365						
Ala	Gly	Phe	Leu	Glu	Gly	Gly	Trp	Glu	Gly	Met	Ile	Ala	Gly	Trp	His	
370					375					380						
Gly	Tyr	Thr	Ser	His	Gly	Ala	His	Gly	Val	Ala	Val	Ala	Ala	Asp	Leu	
385					390					395					400	
Lys	Ser	Thr	Gln	Glu	Ala	Ile	Asn	Lys	Ile	Thr	Lys	Asn	Leu	Asn	Ser	
405					410					415						
Leu	Ser	Glu	Leu	Glu	Val	Lys	Asn	Leu	Gln	Arg	Leu	Ser	Gly	Ala	Met	
420					425					430						
Asp	Glu	Leu	His	Asn	Glu	Ile	Leu	Glu	Leu	Asp	Glu	Lys	Val	Asp	Asp	
435					440					445						
Leu	Arg	Ala	Asp	Thr	Ile	Ser	Ser	Gln	Ile	Glu	Leu	Ala	Val	Leu	Leu	
450					455					460						
Ser	Asn	Glu	Gly	Ile	Ile	Asn	Ser	Glu	Asp	Glu	His	Leu	Leu	Ala	Leu	
465					470					475					480	
Glu	Arg	Lys	Leu	Lys	Lys	Met	Leu	Gly	Pro	Ser	Ala	Val	Asp	Ile	Gly	
485					490					495						
Asn	Gly	Cys	Phe	Glu	Thr	Lys	His	Lys	Cys	Asn	Gln	Thr	Cys	Leu	Asp	
500					505					510						
Arg	Ile	Ala	Ala	Gly	Thr	Phe	Asn	Ala	Gly	Glu	Phe	Ser	Leu	Pro	Thr	
515					520					525						
Phe	Asp	Ser	Leu	Asn	Ile	Thr	Ala	Ala	Ser	Leu	Asn	Asp	Asp	Gly	Leu	
530					535					540						
Asp	Asn	His	Thr	Ile	Leu	Leu	Tyr	Tyr	Ser	Thr	Ala	Ala	Ser	Ser	Leu	
545					550					555					560	
Ala	Val	Thr	Leu	Met	Ile	Ala	Ile	Phe	Ile	Val	Tyr	Met	Val	Ser	Arg	
565					570					575						
Asp	Asn	Val	Ser	Cys	Ser	Ile	Cys	Leu								
580					585											

<400> SEQUENCE: 46

Val His

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Tyr	Asp	Asn	Lys	Gly	Val	Thr	Ala	Lys	Trp	Val	Lys	Lys	Gly	Asn	Ser
1				5					10					15	
His	Ser	Thr	Ser	Ala	Asp	Gln	Ser	Leu	Gln	Ile	Asp	Gln	Glu		
			20					25					30		
<210> SEQ ID NO 52															
<211> LENGTH: 30															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<221> NAME/KEY: source															
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"															
<400> SEQUENCE: 52															
Tyr	Asp	Asn	Lys	Gly	Val	Thr	Ala	Lys	Trp	Val	Lys	Lys	Gly	Asn	Ser
1				5					10					15	
His	Ser	Thr	Thr	Ala	Asp	Gln	Ser	Leu	Gln	Ile	Asp	Gln	Glu		
			20					25					30		
<210> SEQ ID NO 53															
<211> LENGTH: 29															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<221> NAME/KEY: source															
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"															
<400> SEQUENCE: 53															
Tyr	Thr	Thr	Gly	Val	Ser	Ala	Ser	Trp	Thr	Gly	Lys	Asn	Gly	Leu	His
1			5					10					15		
Pro	Asn	Ile	Gly	Asp	Arg	Ala	Leu	His	Lys	Asp	Gln	Glu			
			20					25							
<210> SEQ ID NO 54															
<211> LENGTH: 30															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<221> NAME/KEY: source															
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"															
<400> SEQUENCE: 54															
Tyr	Asp	Asn	Lys	Gly	Val	Thr	Ala	Lys	Trp	Val	Lys	Lys	Gly	Asn	Ser
1				5					10					15	
His	Ser	Thr	Ser	Ala	Asp	Gln	Ser	Leu	Gln	Ile	Glu	Gln	Glu		
			20					25					30		
<210> SEQ ID NO 55															
<211> LENGTH: 29															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<221> NAME/KEY: source															
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"															
<400> SEQUENCE: 55															
Tyr	Thr	Thr	Gly	Val	Ser	Ala	Ser	Trp	Thr	Gly	Lys	Asn	Gly	Leu	His
1			5					10					15		
Pro	Asn	Ile	Gly	Asp	Lys	Ala	Leu	His	Lys	Asp	Gln	Glu			
			20					25							

Thr Lys Val Asn Val Ile Thr
20

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<210> SEQ ID NO 61
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 61

Tyr His His Ser Leu Val Asp Gly Trp Gln Thr Gln Ala Ile Asn Ile
1 5 10 15

Thr Lys Val Asn Val Ile Thr
20

<210> SEQ ID NO 62
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 62

Tyr His His Ser Leu Ile Asp Gly Trp Gln Thr Gln Ala Ile Asn Ile
1 5 10 15

Thr Lys Val Asn Val Ile Thr
20

<210> SEQ ID NO 63
<211> LENGTH: 53
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 63

Asn Ile Thr Ala Ala Ser Ser Gly Arg Ser Leu Val Pro Arg Gly Ser
1 5 10 15

Pro Gly Ser Gly Tyr Ile Pro Glu Ala Pro Arg Asp Gly Gln Ala Tyr
20 25 30

Val Arg Lys Asp Gly Glu Trp Val Leu Leu Ser Thr Phe Leu Gly His
35 40 45

His His His His His
50

<210> SEQ ID NO 64
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 64

Ile Leu Gly Asn Ser Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Lys
20

<210> SEQ ID NO 65
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 65

Ser Tyr Ile Val Glu Thr
20

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 66

Ser Tyr Ile Ala Glu Thr
20

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 67

Ser Tyr Ile Val Glu Thr
20

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 68

Ser Tyr Ile Val Glu Thr
20

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 69

Ser Tyr Ile Val Glu Lys
20

<313> ORGANISM: Influenza virus

100 СПОУПНАСЬ 70

Ser Tyr Ile Ala Glu Thr

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<210> SEQ ID NO 71
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 71

Leu Leu Gly Asn Pro Glu Cys Asp Pro Leu Leu Pro Ala Arg Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Thr
20

<210> SEQ ID NO 72
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 72

Leu Leu Gly Asn Pro Glu Cys Asp Ser Leu Leu Pro Ala Arg Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Thr
20

<210> SEQ ID NO 73
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 73

Leu Leu Gly Asn Pro Glu Cys Asp Pro Leu Leu Pro Val Arg Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Thr
20

<210> SEQ ID NO 74
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 74

Leu Leu Gly Asn Pro Glu Cys Asp Pro Leu Leu Pro Val Arg Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Thr
20

<210> SEQ ID NO 75
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 75

Leu Leu Gly Asn Pro Glu Cys Asp Pro Leu Leu Pro Val Arg Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Thr
20

<210> SEQ ID NO 76
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 76

Leu Leu Gly Asn Pro Glu Cys Asp Ser Leu Leu Pro Ala Arg Ser Trp
1 5 10 15

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<210> SEQ ID NO 82
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Influenza virus
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<400> SEQUENCE: 82

Leu Leu Gly Asn Pro Glu Cys Glu Leu Leu Phe Thr Ala Ser Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Thr
20

<210> SEO ID NO 83

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<211> LENGTH: 22
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<212> TYPE: PRT

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 83

Leu Leu Gly Asn Pro Glu Cys Glu Ser Leu Ser Thr Ala Ser Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Thr
20

<210> SEO ID NO 84

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<211> LENGTH: 22
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<212> TYPE: PRT

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 84

Leu Leu Gly Asn Pro Glu Cys Glu Leu Leu Phe Thr Val Ser Ser Trp
1 5 10 15

Ser Tyr Ile Val Glu Thr
20

<210> SEO ID NO 85

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<211> SEQ ID NO
<211> LENGTH: 6
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<212> TYPE: PRT

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 85

Leu Ile Ser Lys Glu Ser
1 5

<210> SEQ ID NO 86

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<211> LENGTH: 6
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<212> TYPE: PRT

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 86

Leu Leu Pro Ala Ser Ser
1 5

<210> SEQ ID NO 87

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<211> LENGTH: 6
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<212> TYPE: PRT

<213> ORGANISM: Influenza virus

<400> SEQUENCE: 87

Leu Leu Ser Asn Arg Ser
1 5

<210> SEO ID NO 88

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<211> SEQ ID NO
<211> LENGTH: 6
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<211> LENGTH: 8
<212> TYPE: PRT
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<213> ORGANISM: Influenza virus

<400> SEQUENCE: 88

Leu Leu Pro Val Arg Ser

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<210> SEQ ID NO 89 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Influenza virus	
<400> SEQUENCE: 89	
Leu Leu Pro Ala Ser Ser	
1	5
<210> SEQ ID NO 90 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Influenza virus	
<400> SEQUENCE: 90	
Leu Val Ser Lys Glu Ser	
1	5
<210> SEQ ID NO 91 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Influenza virus	
<400> SEQUENCE: 91	
Leu Leu Ser Asn Arg Ser	
1	5
<210> SEQ ID NO 92 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Influenza virus	
<400> SEQUENCE: 92	
Leu Leu Pro Ala Arg Ser	
1	5
<210> SEQ ID NO 93 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Influenza virus	
<400> SEQUENCE: 93	
Leu Leu Pro Ala Arg Ser	
1	5
<210> SEQ ID NO 94 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Influenza virus	
<400> SEQUENCE: 94	
Leu Leu Pro Val Arg Ser	
1	5
<210> SEQ ID NO 95 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Influenza virus	
<400> SEQUENCE: 95	
Leu Leu Pro Val Arg Ser	
1	5

<210> SEQ ID NO 96	
<211> LENGTH: 6	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 96	
Leu Leu Pro Val Arg Ser	
1 5	
<210> SEQ ID NO 97	
<211> LENGTH: 6	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 97	
Leu Leu Pro Ala Arg Ser	
1 5	
<210> SEQ ID NO 98	
<211> LENGTH: 6	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 98	
Leu Leu Pro Ala Arg Ser	
1 5	
<210> SEQ ID NO 99	
<211> LENGTH: 6	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 99	
Leu Leu Thr Val Ser Ser	
1 5	
<210> SEQ ID NO 100	
<211> LENGTH: 6	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 100	
Leu Leu Thr Val Ser Ser	
1 5	
<210> SEQ ID NO 101	
<211> LENGTH: 6	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 101	
Leu Phe Thr Ala Ser Ser	
1 5	
<210> SEQ ID NO 102	
<211> LENGTH: 6	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 102	
Leu Phe Thr Ala Ser Ser	
1 5	
<210> SEQ ID NO 103	

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<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 103

Leu Phe Thr Ala Ser Ser
1 5

<210> SEQ ID NO 104
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 104

Leu Ser Thr Ala Ser Ser
1 5

<210> SEQ ID NO 105
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 105

Leu Phe Thr Val Ser Ser
1 5

<210> SEQ ID NO 106
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 106

Leu Ile Ser Lys Glu Ser
1 5

<210> SEQ ID NO 107
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 107

Leu Leu Pro Ala Ser Ser
1 5

<210> SEQ ID NO 108
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 108

Leu Leu Ser Asn Arg Ser
1 5

<210> SEQ ID NO 109
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 109

Leu Leu Pro Val Arg Ser
1 5

<210> SEQ ID NO 110
<211> LENGTH: 6
<212> TYPE: PRT

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<213> ORGANISM:	Influenza virus
<400> SEQUENCE:	110
Leu Val Ser Lys Glu Ser	
1	5
<210> SEQ ID NO	111
<211> LENGTH:	6
<212> TYPE:	PRT
<213> ORGANISM:	Influenza virus
<400> SEQUENCE:	111
Leu Leu Pro Ala Arg Ser	
1	5
<210> SEQ ID NO	112
<211> LENGTH:	6
<212> TYPE:	PRT
<213> ORGANISM:	Influenza virus
<400> SEQUENCE:	112
Leu Leu Thr Val Ser Ser	
1	5
<210> SEQ ID NO	113
<211> LENGTH:	6
<212> TYPE:	PRT
<213> ORGANISM:	Influenza virus
<400> SEQUENCE:	113
Leu Phe Thr Ala Ser Ser	
1	5
<210> SEQ ID NO	114
<211> LENGTH:	6
<212> TYPE:	PRT
<213> ORGANISM:	Influenza virus
<400> SEQUENCE:	114
Leu Ser Thr Ala Ser Ser	
1	5
<210> SEQ ID NO	115
<211> LENGTH:	6
<212> TYPE:	PRT
<213> ORGANISM:	Influenza virus
<400> SEQUENCE:	115
Leu Phe Thr Val Ser Ser	
1	5
<210> SEQ ID NO	116
<211> LENGTH:	6
<212> TYPE:	PRT
<213> ORGANISM:	Artificial Sequence
<220> FEATURE:	
<221> NAME/KEY:	source
<223> OTHER INFORMATION:	/note="Description of Artificial Sequence: Synthetic peptide"
<400> SEQUENCE:	116
Leu Ile Ser Lys Glu Ser	
1	5
<210> SEQ ID NO	117

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<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 117

Leu Leu Pro Val Arg Ser
1 5

<210> SEQ ID NO 118
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 118

Leu Leu Thr Val Ser Ser
1 5

<210> SEQ ID NO 119
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 119

Leu Leu Pro Ala Ser Ser
1 5

<210> SEQ ID NO 120
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 120

Leu Val Ser Lys Glu Ser
1 5

<210> SEQ ID NO 121
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 121

Leu Phe Thr Ala Ser Ser
1 5

<210> SEQ ID NO 122
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 122

Leu Leu Ser Asn Arg Ser
1 5

<210> SEQ ID NO 123
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 123

Leu Leu Pro Ala Arg Ser
1 5

<210> SEQ ID NO 124
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 124

Leu Ser Thr Ala Ser Ser
1 5

<210> SEQ ID NO 125
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 125

Leu Leu Pro Val Arg Ser
1 5

<210> SEQ ID NO 126
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 126

Leu Leu Thr Val Ser Ser
1 5

<210> SEQ ID NO 127
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

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<400> SEQUENCE: 127									
Leu	Phe	Thr	Val	Ser	Ser				
1				5					
<210> SEQ ID NO 128									
<211> LENGTH: 6									
<212> TYPE: PRT									
<213> ORGANISM: Artificial Sequence									
<220> FEATURE:									
<221> NAME/KEY: source									
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"									
<400> SEQUENCE: 128									
Leu	Val	Ser	Lys	Glu	Ser				
1				5					
<210> SEQ ID NO 129									
<211> LENGTH: 6									
<212> TYPE: PRT									
<213> ORGANISM: Artificial Sequence									
<220> FEATURE:									
<221> NAME/KEY: source									
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"									
<400> SEQUENCE: 129									
Leu	Phe	Thr	Ala	Ser	Ser				
1				5					
<210> SEQ ID NO 130									
<211> LENGTH: 6									
<212> TYPE: PRT									
<213> ORGANISM: Artificial Sequence									
<220> FEATURE:									
<221> NAME/KEY: source									
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"									
<400> SEQUENCE: 130									
Leu	Leu	Pro	Val	Arg	Ser				
1				5					
<210> SEQ ID NO 131									
<211> LENGTH: 11									
<212> TYPE: PRT									
<213> ORGANISM: Artificial Sequence									
<220> FEATURE:									
<221> NAME/KEY: source									
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"									
<400> SEQUENCE: 131									
Val	Asn	Asn	Lys	Glu	Ser	Ser	Asn	Glu	Pro Gly
1				5				10	
<210> SEQ ID NO 132									
<211> LENGTH: 8									
<212> TYPE: PRT									
<213> ORGANISM: Artificial Sequence									
<220> FEATURE:									
<221> NAME/KEY: source									
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"									
<400> SEQUENCE: 132									
Ser	His	Ala	Arg	Lys	Ser	Arg	Asn		
1				5					

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<210> SEQ ID NO 133
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 133

Leu Leu Pro Ala Arg Ser Trp Ser
1 5

<210> SEQ ID NO 134
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 134

Leu Leu Pro Ala Arg Ser Trp Ser
1 5

<210> SEQ ID NO 135
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 135

Glu Ile Phe Pro
1

<210> SEQ ID NO 136
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 136

Ser Trp Pro Asn
1

<210> SEQ ID NO 137
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic peptide"

<400> SEQUENCE: 137

Ser His Ala Arg Lys Ser
1 5

<210> SEQ ID NO 138
<211> LENGTH: 11

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
        Synthetic peptide"
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<400> SEQUENCE: 138

Ile Asn Asp Lys Gly Thr Ser Arg Glu Pro Gly
1 5 10

```
<210> SEQ ID NO 139
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
      Synthetic peptide"
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<400> SEQUENCE: 139

Pro His Ala Gly Ala Lys Arg Asp
1 5

```
<210> SEQ ID NO 140
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
      Synthetic peptide"
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<400> SEQUENCE: 140

Leu Ser Thr Ala Ser Ser Trp Ser
1 5

```
<210> SEQ ID NO 141
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
      Synthetic peptide"
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<400> SEQUENCE: 141

Leu Ser Thr Ala Ser Ser Trp Ser
1 5

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<210> SEQ ID NO 142
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
      Synthetic peptide"
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<400> SEQUENCE: 142

Glu Ile Phe Pro
1

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<210> SEQ ID NO 143
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
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<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"	
<400> SEQUENCE: 143	
Ser Trp Pro Asn 1	
<210> SEQ ID NO 144 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <221> NAME/KEY: source <223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"	
<400> SEQUENCE: 144	
Pro His Ala Gly Ala Lys 1 5	
<210> SEQ ID NO 145 <211> LENGTH: 11 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <221> NAME/KEY: source <223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"	
<400> SEQUENCE: 145	
Val Asn Asn Lys Glu Ser Ser Asn Glu Pro Gly 1 5 10	
<210> SEQ ID NO 146 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <221> NAME/KEY: source <223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"	
<400> SEQUENCE: 146	
Ser His Ala Arg Lys Ser Arg Asn 1 5	
<210> SEQ ID NO 147 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <221> NAME/KEY: source <223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"	
<400> SEQUENCE: 147	
Leu Leu Pro Ala Arg Ser Trp Ser 1 5	
<210> SEQ ID NO 148 <211> LENGTH: 72 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <221> NAME/KEY: source <223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"	
<400> SEQUENCE: 148	

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Leu	Leu	Pro	Ala	Arg	Ser	Trp	Ser	Tyr	Ile	Val	Glu	Thr	Pro	Asn	Pro	
1				5					10					15		
Glu	Asn	Gly	Thr	Cys	Tyr	Pro	Gly	Tyr	Phe	Ala	Asp	Tyr	Glu	Glu	Leu	
			20					25					30			
Arg	Glu	Gln	Leu	Ser	Ser	Val	Ser	Ser	Phe	Glu	Arg	Phe	Glu	Ile	Phe	
			35				40					45				
Pro	Lys	Glu	Ser	Ser	Trp	Pro	Asn	His	Glu	Thr	Asn	Gly	Val	Ser	Ala	
	50					55					60					
Ser	Cys	Ser	His	Ala	Arg	Lys	Ser									
65					70											
<210> SEQ ID NO 149																
<211> LENGTH: 11																
<212> TYPE: PRT																
<213> ORGANISM: Artificial Sequence																
<220> FEATURE:																
<221> NAME/KEY: source																
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"																
<400> SEQUENCE: 149																
Ile	Asn	Asp	Lys	Gly	Thr	Ser	Arg	Glu	Pro	Gly						
1				5					10							
<210> SEQ ID NO 150																
<211> LENGTH: 8																
<212> TYPE: PRT																
<213> ORGANISM: Artificial Sequence																
<220> FEATURE:																
<221> NAME/KEY: source																
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"																
<400> SEQUENCE: 150																
Pro	His	Ala	Gly	Ala	Lys	Arg	Asp									
1				5												
<210> SEQ ID NO 151																
<211> LENGTH: 8																
<212> TYPE: PRT																
<213> ORGANISM: Artificial Sequence																
<220> FEATURE:																
<221> NAME/KEY: source																
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic peptide"																
<400> SEQUENCE: 151																
Leu	Ser	Thr	Ala	Ser	Ser	Trp	Ser									
1				5												
<210> SEQ ID NO 152																
<211> LENGTH: 72																
<212> TYPE: PRT																
<213> ORGANISM: Artificial Sequence																
<220> FEATURE:																
<221> NAME/KEY: source																
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic polypeptide"																
<400> SEQUENCE: 152																
Leu	Ser	Thr	Ala	Ser	Ser	Trp	Ser	Tyr	Ile	Val	Glu	Thr	Pro	Asn	Pro	
1				5					10					15		
Glu	Asn	Gly	Thr	Cys	Tyr	Pro	Gly	Tyr	Phe	Ala	Asp	Tyr	Glu	Glu	Leu	
			20					25					30			

Arg Glu Gln Leu Ser Ser Val Ser Ser Phe Glu Arg Phe Glu Ile Phe
35 40 45

Pro Lys Glu Ser Ser Trp Pro Asn His Thr Val Thr Gly Val Ser Ala
50 55 60

Ser Cys Pro His Ala Gly Ala Lys
65 70

```
<210> SEQ ID NO 153
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Influenza virus
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<400> SEQUENCE: 153

Val Asp Gly Trp
1

```
<210> SEQ ID NO 154
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Influenza virus
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<400> SEQUENCE: 154

Ser Ser Pro Asn Lys Gly Ser Ser Tyr Pro Lys Ser Lys Ser Val
 1 5 10 15

```
<210> SEQ ID NO 155
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Influenza virus
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<400> SEQUENCE: 155

Ile Ile Pro Lys Ser Ser Trp Ser
1 5

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<210> SEQ ID NO 156
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Influenza virus
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<400> SEQUENCE: 156

Ile Ile Trp Glu Ser Lys Asn
1 5

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<210> SEQ ID NO 157
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Influenza virus
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<400> SEQUENCE: 157

Gln Ile Ile Pro Glu Ala Ser Leu
1 5

```
<210> SEQ ID NO 158
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Influenza virus
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<400> SEQUENCE: 158

Ser Trp Ser Tyr Asn Asn Thr
1 5

```
<210> SEQ ID NO 159
<211> LENGTH: 9
<212> TYPE: PRT
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<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 159	
Asn Phe Asp Lys Leu Tyr Ile Trp Gly	
1 5	
<210> SEQ ID NO 160	
<211> LENGTH: 13	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 160	
Pro Asn Lys Lys Gly Asn Ser Pro Lys Leu Ser Lys Ser	
1 5 10	
<210> SEQ ID NO 161	
<211> LENGTH: 5	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 161	
Val Asn Asn Lys Glu	
1 5	
<210> SEQ ID NO 162	
<211> LENGTH: 66	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 162	
Phe Glu Ile Phe Pro Lys Thr Ser Ser Trp Pro Asn His Glu Thr Thr	
1 5 10 15	
Lys Gly Val Thr Ala Ala Cys Ser Tyr Ala Gly Ala Ser Ser Phe Tyr	
20 25 30	
Arg Asn Leu Leu Trp Leu Thr Lys Lys Gly Ser Ser Tyr Pro Lys Leu	
35 40 45	
Ser Lys Ser Tyr Val Asn Asn Lys Gly Lys Glu Val Leu Val Leu Trp	
50 55 60	
Gly Val	
65	
<210> SEQ ID NO 163	
<211> LENGTH: 62	
<212> TYPE: PRT	
<213> ORGANISM: Influenza virus	
<400> SEQUENCE: 163	
Asn Asn Glu Ser Phe Asn Trp Thr Gly Val Thr Gln Asn Gly Thr Ser	
1 5 10 15	
Ser Ala Cys Lys Arg Arg Ser Asn Asn Ser Phe Phe Ser Arg Leu Asn	
20 25 30	
Trp Leu Thr Gln Leu Lys Phe Lys Tyr Pro Ala Leu Lys Val Thr Met	
35 40 45	
Pro Asn Asn Glu Lys Phe Asp Lys Leu Tyr Ile Trp Gly Val	
50 55 60	

- We claim:
1. A method of producing an engineered mosaic influenza B hemagglutinin (HA) polypeptide, comprising:
a) aligning influenza B HA amino acid sequences from multiple circulating strains of influenza B virus to generate an alignment;
- b) identifying the positions of amino acids corresponding to known B cell epitopes and antigenic regions;
c) compiling amino acid residues across the alignment at the identified positions for each B cell epitope and antigenic region, thereby generating an antigenic repertoire for each B cell epitope and antigenic region;

199

- d) defining a set of amino acid sequence patterns within the antigenic repertoire for each B cell epitope and antigenic region, wherein each amino acid sequence pattern in the set is represented only once;
- e) i) selecting a sequence from the set of amino acid sequence patterns for each B cell epitope or antigenic region, thereby resulting in a combination of selected sequences, each selected sequence corresponding to a different B cell epitope or antigenic region, or ii) generating a consensus sequence from each set for each B cell epitope or antigenic region, thereby resulting in a combination of consensus sequences, each consensus sequence corresponding to a different B cell epitope or antigenic region;
- f) inserting the combination of selected sequences or the combination of consensus sequences into corresponding locations in a structural backbone of influenza B HA, thereby generating a mosaic influenza B HA polypeptide sequence;
- g) repeating steps e) and f) to generate a population of mosaic influenza B HA polypeptide sequences, wherein each mosaic influenza B HA polypeptide sequence in the population is different from one another;
- h) calculating folding energy of each mosaic influenza B HA polypeptide sequence in the population and selecting one with a negative total energy score as the engineered mosaic influenza B HA polypeptide, wherein the engineered mosaic influenza B HA polypeptide is distinct from naturally circulating strains; and
- i) expressing the engineered mosaic influenza B HA polypeptide in a host cell transformed with an expression vector comprising a nucleic acid molecule encoding the engineered mosaic influenza B HA polypeptide.
- 2.** The method of claim 1, wherein the influenza B virus is a Yamagata lineage strain, a Victoria lineage strain, or type B influenza virus selected from B/Hong Kong/330/2001, B/Hong Kong/05/1972, B/Lee/40, B/Massachusetts/02/2012, B/Panama/45/1990, B/Singapore/222/79, B/Victoria/02/1987, B/Yamagata/16/1988, or B/Brisbane/60/2008.
- 3.** The method of claim 1, wherein: i) the corresponding locations in the structural backbone in step f) do not overlap; or ii) the steps are performed in silico by a suitably programmed computer system.
- 4.** The method of claim 1, wherein the selecting step further comprises ranking the selected sequences by sequence identity, geographical location and/or isolation date of the influenza B virus.
- 5.** The method of claim 1, wherein the selecting step is random.
- 6.** The method of claim 1, wherein the sequences are selected so the engineered mosaic influenza B HA polypeptide elicits a broadly neutralizing immune response against the multiple circulating influenza strains.
- 7.** The method of claim 6, wherein the sequences are selected according to a predetermined algorithm.
- 8.** The method of claim 1, wherein prior to the selecting step, the amino acid sequence patterns in the set are weighted by their frequency of occurrence.
- 9.** The method of claim 8, wherein the selecting step comprises selecting the most frequently occurring sequence for each of the B cell epitopes or antigenic regions, the second most frequently occurring sequence for each of the B cell epitopes or antigenic regions, or the third most frequently occurring sequence for each of the B cell epitopes or antigenic regions.

200

10. The method of claim 1, wherein:

- a) the aligning step comprises identifying redundant sequences by screening the influenza B HA amino acid sequences for those with greater than 99.9% sequence identity, and removing all but one of the redundant sequences; and/or
- b) the aligning step comprises obtaining 100-5000 influenza B HA amino acid sequences from multiple circulating influenza B strains; and/or
- c) the aligning step comprises obtaining all publicly available influenza B HA amino acid sequences.

11. The method of claim 1, further comprising:

- generating a nucleic acid sequence encoding the engineered mosaic influenza B HA polypeptide;
- cloning the nucleic acid sequence into a mammalian expression vector; and
- transfecting a mammalian host cell with the mammalian expression vector.

12. The method of claim 11, wherein the mammalian host cell is a Vero cell.

13. The method of claim 1, wherein the B cell epitopes are neutralizing epitopes and/or discontinuous epitopes.

14. The method of claim 1, wherein the engineered mosaic influenza B HA polypeptide comprises a mosaic amino acid sequence pattern comprising one or more amino acid substitutions at residues 21, 22, 23, 26, 37, 38, 43, 45, 46, 47, 52, 53, 54, 55, 56, 58, 60, 62, 63, 67, 70, 73, 74, 75, 76, 77, 85, 86, 89, 90, 91, 92, 93, 94, 95, 100, 101, 102, 103, 104, 105, 106, 110, 128, 130, 131, 133, 136, 137, 138, 140, 141, 143, 145, 146, 153, 154, 155, 156, 159, 160, 161, 162, 165, 173, 174, 175, 176, 177, 179, 180, 181, 195, 196, 197, 198, 199, 200, 209, 210, 211, 212, 213, 214, 215, 216, 217, 225, 227, 228, 245, 248, 250, 251, 257, 258, 259, 277, 279, 290, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 314, 315, 319, 320, 321, 322, 323, 348, 354, 355, 356, 377, 378, 380, 381, 382, 383, 387, 392, 394, 395, 396, 397, 398, 400, 401, 403, 404, 405, 406, 407, 408, 410, 411, 412, 413, 414, 415, 417, 418, 419, 420, 421, 422, 470, 508, and/or 512 as indexed by reference to a B/Brisbane/60/2008 HA.

15. The method of claim 14, wherein the engineered mosaic influenza B HA polypeptide comprises a mosaic amino acid sequence pattern comprising one or more amino acid substitutions at residues 21G, 22I, 23T, 26N/K, 37G, 38E, 43G, 45I, 46P/S, 47L, 52T, 53K, 54S, 55Y/H, 56F, 58N, 60K/R, 62T, 63E/K, 67K, 70P, 73L/P, 74N, 75C, 76T, 77D, 85P, 86K/M, 89G, 90K/T/N, 91I/T, 92P, 93S, 94A, 95R/K, 100H, 101E, 102V, 103R, 104P, 105V, 106T, 110F, 128G, 130E, 131H, 133R, 136T/N, 137Q/H/Y, 138N, 140I, 141D/N, 143E, 145A, 146P, 153G, 154T, 155S, 156G/R, 159P, 160N, 161A/V, 162T, 165S/I/N, 165K/R, 173W, 174A, 175V, 176P, 177K, 179D, 180N/S, 181N, 195I, 196C, 197T/A, 198E, 199G, 200E, 209H, 210S, 211D, 212N/D, 213K, 214T, 215Q, 216M, 217K/V, 225P, 227K, 228F, 245G/D, 248D/N, 250T, 251E, 257Q, 258S, 259G, 277V/T, 279Q, 290C, 300G, 301 S/T, 302L, 303P, 304L, 305I, 306G, 307E, 308A, 309D, 314K, 315Y, 319N, 320K, 321S, 322K, 323P, 348N, 354P, 355P, 356A/T, 377E, 378G, 380V/I, 381A, 382G, 383W, 387T, 392H, 394V, 395A, 396V, 397A, 398A, 400L, 401K, 403T, 404Q, 405E, 406A, 407I, 408N, 410I, 411T, 412K, 413N, 414L, 415N, 417L, 418S, 419E, 420L, 421E, 422I/V, 470I, 508Q, and/or 512D/N as indexed by reference to a B/Brisbane/60/2008 HA.

16. The method of claim 1, wherein the engineered mosaic influenza B HA polypeptide comprises a mosaic amino acid sequence pattern defined by amino acid substitutions 131H and 213K as indexed by reference to a B/Brisbane/60/2008 amino acid sequence.

17. The method of claim 16, wherein the engineered mosaic influenza B HA polypeptide further comprises a mosaic amino acid sequence pattern defined by 159P, 160N, 161A, 162T, 163N, 164K and 165S as indexed by reference to a B/Brisbane/60/2008 HA. 5

18. The method of claim 1, wherein the structural backbone of influenza B HA is from an influenza B virus of a Yamagata lineage strain or a Victoria lineage strain, or a type B influenza virus selected from:
CAA25425|HA|Human|fluB|B/Singapore/222/79|Singapore|1979|, 10
AGL06036|HA|Human|fluB|B/Massachusetts/02/2012|USA|2012/03/13|,
ABL76694|HA|Human|fluB|B/Panama/45/1990|Panama|1990/03/7|, or 15
AFH57909|HA|Human|fluB|B/Brisbane/60/2008|Australia|2008|.

19. The method of claim 1, wherein the engineered mosaic influenza B HA polypeptide comprises:
a) an amino acid sequence at least 98% identical to SEQ ID NO: 40; 20
b) an amino acid sequence at least 98% identical to SEQ ID NO: 41;
c) an amino acid sequence at least 97% identical to SEQ ID NO: 42; 25
d) an amino acid sequence at least 98% identical to SEQ ID NO: 43;
e) an amino acid sequence at least 97% identical to SEQ ID NO: 44; or
f) an amino acid sequence at least 97% identical to SEQ ID NO: 45. 30

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